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THIO DERIVATIVES OF HYDROXAMIC ACIDS

Abstract:

Abstract of WO 9742168

(A1) Compounds of formula (I), wherein R<1> is an aryl, arylC1-6alkyl, heteroaryl or heteroarylC1-6alkyl group; R<2> is hydrogen, C1-8alkyl, C2-6alkenyl, C2-6alkynyl, C3-8cycloalkyl, heteroaryl, heterocyclyl, arylC1-6alkyl, heteroarylC1-6alkyl, heterocyclylC1-6alkyl or C3-8cycloalkylC1-6alkyl; R<3> is C1-6alkyl, C2-6alkenyl, aryl, C1-6alkyl, heteroarylC1-6alkyl or the side-chain of a naturally occurring amino acid; R<4> is hydrogen, C1-6alkyl, C3-8cycloalkyl, C4-8cycloalkenyl, arylC1-6alkyl, heteroarylC1-6alkyl or heterocyclylC1-6alkyl; R<5> is hydrogen or C1-6alkyl; or R<4> and R<5> together with the nitrogen atom to which they are joined form a heterocyclic ring; wherein any group or ring, in R<1>-R<5>, is optionally substituted; or pharmaceutically acceptable salts or in vivo hydrolysable esters thereof, are described as inhibitors of the production of Tumour Necrosis Factor and/or one or more matrix metalloproteinase enzymes. Compositions containing them and their preparation are also described.

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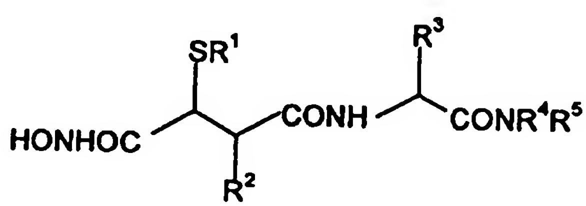

(I)

(57) Abstract

Compounds of formula (I), wherein R^1 is an aryl, arylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6}alkyl group; R^2 is hydrogen, C_{1-8}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{3-8}cycloalkyl, heteroaryl, heterocyclyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl, heterocyclylC_{1-6}alkyl or C_{3-8}cycloalkylC_{1-6}alkyl; R^3 is C_{1-6}alkyl, C_{2-6}alkenyl, aryl, C_{1-6}alkyl, heteroarylC_{1-6}alkyl or the side-chain of a naturally occurring amino acid; R^4 is hydrogen, C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{4-8}cycloalkenyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl or heterocyclylC_{1-6}alkyl; R^5 is hydrogen or C_{1-6}alkyl; or R^4 and R^5 together with the nitrogen atom to which they are joined form a heterocyclic ring; wherein any group or ring, in R^1-R^5, is optionally substituted; or pharmaceutically acceptable salts or *in vivo* hydrolysable esters thereof, are described as inhibitors of the production of Tumour Necrosis Factor and/or one or more matrix metalloproteinase enzymes. Compositions containing them and their preparation are also described.

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THIO DERIVATIVES OF HYDROXAMIC ACIDS

This invention relates to thio compounds and in particular to thio compounds wherein a thio substituent is located adjacent to a hydroxycarbamate group. This invention further relates to processes for preparing such thio compounds, to pharmaceutical compositions containing them and to their use in methods of therapeutic treatment.

The compounds of this invention are inhibitors of the production of TNF (Tumour Necrosis Factor) which is believed to be formed by the cleavage of a pro-form, or larger precursor, by the enzyme pro-TNF Convertase. Applicants believe that the compounds of the present invention inhibit TNF production by mechanisms which include inhibition of pro-TNF Convertase. The term 'TNF' is used herein to refer to Tumour Necrosis Factor in general but, in particular, to TNFα.

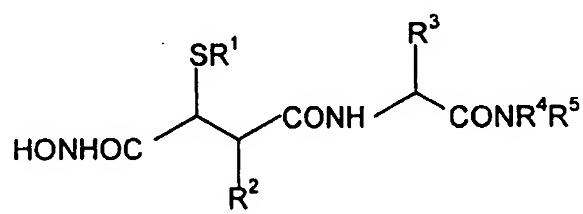
The compounds of this invention will be useful in the treatment of disease or medical conditions in which excessive TNF production is known to give rise via a cascade of processes to a variety of physiological sequelae including the production of physiologically-active eicosanoids such as the prostaglandins and leukotrienes, the stimulation of the release of proteolytic enzymes such as collagenase, the activation of osteoclast activity leading to the resorption of calcium, the stimulation of the release of proteoglycans from, for example, cartilage, the stimulation of cell proliferations and to angiogenesis. It is also known that, in certain cellular systems, TNF production precedes and mediates the production of other cytokines such as interleukin-1 (IL-1) and interleukin-2 (IL-2) which are also believed to contribute to the pathology of disease states such as inflammatory and allergic diseases and cytokine-induced toxicity. Excessive TNF production has also been implicated in mediating or exacerbating the development of various inflammatory and allergic diseases such as inflammation of the joints (especially rheumatoid arthritis, osteoarthritis and gout), inflammation of the gastrointestinal tract (especially inflammatory bowel disease, ulcerative colitis and gastritis), skin disease (especially psoriasis, eczema and dermatitis) and respiratory disease (especially asthma, bronchitis and allergic rhinitis), and in the production and development of various cardiovascular disorders such as myocardial infarction, angina and peripheral vascular disease. Excessive TNF production has also been implicated in mediating complications of bacterial, fungal and/or viral infections such as endotoxic shock, septic

shock and toxic shock syndrome. Excessive TNF production has also been implicated in mediating or exacerbating the development of adult respiratory distress syndrome, diseases involving cartilage or muscle resorption, Paget's disease and osteoporosis, pulmonary fibrosis, cirrhosis, renal fibrosis, the cachexia found in certain chronic diseases such as malignant disease and acquired immune deficiency syndrome (AIDS), tumour invasiveness and tumour metastasis and multiple sclerosis.

The compounds of the invention may also be inhibitors of one or more matrix metalloproteinases such as collagenases, stromelysins and gelatinases. Thus they may also be of use in the therapeutic treatment of disease conditions mediated by such enzymes for example arthritis (rheumatoid and osteoarthritis), osteoporosis and tumour metastasis.

The present invention provides novel thio compounds which have activity as inhibitors of TNF production and/or are inhibitors of one or more matrix metalloproteinase enzymes.

Accordingly the present invention provides a compound of the formula (I):



(I)

wherein:

R^1 is is aryl, arylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6} alkyl;

R^2 is is hydrogen, C_{1-8}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{3-8}cycloalkyl, aryl, heteroaryl, heterocyclyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl, heterocyclylC_{1-6}alkyl or C_{3-8}cycloalkylC_{1-6}alkyl;

R^3 is C_{1-6}alkyl, C_{2-6}alkenyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl or the side-chain of a naturally occurring amino acid;

R^4 is hydrogen, C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{4-8}cycloalkenyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl or heterocyclylC_{1-6}alkyl;

R^5 is hydrogen or C_{1-6}alkyl; or R^4 or R^5 together with the nitrogen atom to which they are joined form a heterocyclic ring;

wherein any group or ring, in R^1-R^5, is optionally substituted;
or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof.

"Aryl in the terms "aryl" and "arylC_{1-6}alkyl" typically means phenyl or naphthyl, preferably phenyl. "Heteroaryl" in the terms "heteroaryl" and "heteroarylC_{1-6}alkyl" means an aromatic mono- or bicyclic 5-10 membered ring with up to five ring heteroatoms selected from nitrogen, oxygen and sulphur. Examples of 'heteroaryl' include thienyl, pyrrolyl, furanyl, imidazolyl, thiazolyl, pyrimidinyl, pyridinyl, indolyl, benzimidazolyl, benzthiazolyl, quinolinyl and isoquinolinyl. "Heterocyclyl" in the terms "heterocyclyl" and heterocyclyl-C_{1-6}alkyl means a non-aromatic mono- or bicyclic 5-10 membered ring with up to five ring hetero atoms selected from nitrogen, oxygen and sulphur. Examples of 'heterocyclyl' include pyrrolidinyl, morpholinyl, piperidinyl, dihydropyridinyl and dihydropyrimidinyl.

Any group or ring in R^1-R^5 may be optionally substituted, for example by up to three substituents which may be the same or different. Typical substituents include: hydroxy, C_{1-6}alkoxy for example methoxy, mercapto, C_{1-6}alkylthio for example methylthio, amino, C_{1-6}alkylamino for example methylamino, di-(C_{1-6}alkyl)amino for example dimethylamino, carboxy, carbamoyl, C_{1-6}alkylcarbamoyl for example methylcarbamoyl, di-C_{1-6}alkylcarbamoyl for example dimethylcarbamoyl, C_{1-6}alkylsulphonyl for example methylsulphonyl, arylsulphonyl for example phenylsulphonyl, C_{1-6}alkylaminosulphonyl for example methylaminosulphonyl, di-(C_{1-6}alkyl)aminosulphonyl for example dimethylamino-sulphonyl, nitro, cyano, cyanoC_{1-6}alkyl for example cyanomethyl, hydroxyC_{1-6}alkyl for example hydroxymethyl, aminoC_{1-6}alkyl for example aminoethyl, C_{1-6}alkanoylamino for example acetamido, C_{1-6}alkoxycarbonylamino for example methoxycarbonylamino, C_{1-6}alkanoyl for example acetyl, C_{1-6}alkanoyloxy for example acetoxy, C_{1-6}alkyl for example methyl, ethyl, isopropyl or tert-butyl, halo for example fluoro, chloro or bromo, trifluoromethyl, aryl for example phenyl, arylC_{1-6}alkyl for example benzyl, aryloxy for example phenoxy, arylC_{1-6}alkoxy for example benzyloxy, heteroaryl, heteroarylC_{1-6}alkyl, heterocyclyl and heterocyclylC_{1-6}alkyl. The term "side chain of a naturally occurring amino acid" means the side chain X of an amino acid NH_2-CHX-COOH. Suitable amino acids include alanine, arginine, aspartic acid, cysteine, asparagine, glutamine, histidine, homoserine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, serine, threonine, tryptophan, tyrosine and valine.

The compounds of the present invention possess a number of chiral centres, at -CH(SR^1)-, at -CHR^3-, at -CHR^2- (when R^2 is not hydrogen) and possibly in the variables R^1-R^5. The present invention covers all diastereoisomers and mixtures thereof that inhibit pro-TNF Convertase and/or inhibit matrix metalloproteinase enzymes.

In one aspect R^1 is an optionally substituted aryl group. Suitably R^1 is an optionally substituted phenyl group. In another aspect R^1 is optionally substituted arylC$_{1-6}$alkyl. In yet a further aspect R^1 is optionally substituted heteroaryl or heteroaryl-C$_{1-6}$alkyl.

Favourably R^1 is phenyl, phenylC$_{1-6}$alkyl, naphthylC$_{1-6}$alkyl, heteroaryl or heteroarylC$_{1-6}$alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C$_{1-6}$alkylcarbonyl for example acetyl, C$_{1-6}$alkylsulphonyl for example methylsulphonyl, trifluoromethyl, cyano, C$_{1-6}$alkyl for example methyl, isopropyl or tert-butyl, C$_{1-6}$alkoxy for example methoxy, cyanoC$_{1-6}$alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on a phenyl ring are linked to form a methylenedioxy (-OCH$_2$O-) group.

In another aspect R^1 is phenyl, phenylC$_{1-6}$alkyl, naphthylC$_{1-6}$alkyl, heteroaryl or heteroarylC$_{1-6}$alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C$_{1-6}$alkylcarbonyl for example acetyl, C$_{1-6}$alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C$_{1-6}$alkyl for example methyl, isopropyl or tert-butyl, C$_{1-6}$alkoxy for example methoxy, cyanoC$_{1-6}$alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on a phenyl ring are linked to form a methylenedioxy (-OCH$_2$O-) group.

Further examples within the meaning of R^1 as an optionally substituted aryl group include those wherein two adjacent carbon atoms on a phenyl ring are linked by -(CH$_2$)$_m$- wherein m is 3 or 4, by -NR^a-CO-(CH$_2$)$_n$- wherein R^a is hydrogen or C$_{1-6}$alkyl and n is 1 or 2, by -NR^a-COCH=CH-, -CO-NR^a-(CH$_2$)$_n$- or by -CONR^a-CH=CH-.

Preferably R^1 is phenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 3,5-difluorophenyl, 4-acetylphenyl, 4-cyanophenyl, 4-methylsulphonylphenyl, 4-(1-cyano-1-methylethyl)phenyl, 3,4-dimethoxyphenyl, 3,5-dichlorophenyl or 3,5-di-trifluoromethylphenyl. Preferably also R^1 is 3-(1-cyano-1-methylethyl)phenyl, naphth-1-yl, 3-hydroxynaphth-7-yl or 2-chloro-4-fluorophenyl.

In a particular aspect R^1 is phenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 3,5-difluoro-phenyl, 4-acetylphenyl, 4-methylsulphonylphenyl, 4-(1-cyano-1-methylethyl)phenyl, 3,4-dimethoxyphenyl or 3,5-dichlorophenyl.

In particular R^1 may also be benzyl, phenethyl, phenylprop-1-yl, 1-methyl-phenylmethyl (PhCHMe-), 1,1-dimethylphenylmethyl ($PhCMe_2$-), thiazolyl, benzthiazolyl, 4-methoxybenzyl, indolyl, benzimidazolyl, indolylmethyl, pyrimidinyl, quinolinyl for example quinolin-2-yl, quinolin-6-yl or quinolin-7-yl, isoquinolinyl, pyridinyl or quinolinylmethyl for example quinolin-8-ylmethyl. Of these benzthiazolyl, quinolin-2-yl, quinolin-8-ylmethyl and benzyl are preferred.

In a particular aspect R^1 may be benzyl, phenethyl, phenylprop-1-yl, 1-methyl-phenylmethyl (PhCHMe-), 1,1-dimethylphenylmethyl ($PhCMe_2$-), thiazolyl, benzthiazolyl, 4-methoxybenzyl, indolyl, benzimidazolyl or indolylmethyl.

Particularly also R^1 may be 1-methyl-2-oxo-quinolin-6-yl, 1-methyl-2-oxodihydro-quinolinyl, 1-methyl-2-oxotetrahydroquinolinyl, 2-methyl-1-oxodihydroisoquinolinyl or 2-methyl-1-oxotetrahydroisoquinolinyl; of these 1-methyl-2-oxotetrahydroquinolin-7-yl is preferred.

Particular groups for R^2 include C_{1-8}alkyl for example isopropyl, n-propyl, isobutyl, sec-butyl, n-butyl, tert-butyl, isopentyl, n-pentyl, hexyl, heptyl or octyl; C_{1-8}alkyl interrupted by an oxygen or sulphur atom for example methoxypropyl, ethoxyethyl, propoxymethyl, ethylthioethyl, methylthiopropyl; phenylC_{1-6}alkyl for example benzyl, phenethyl, phenylpropyl or phenylbutyl; phenylC_{1-6}alkyl wherein the alkyl chain is interrupted by oxygen or sulphur for example benzyloxypropyl and benzyloxybutyl; C_{3-8}cycloalkyl for example cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl; or C_{3-8}cycloalkylC_{1-6}alkyl for example cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyL

In a particular aspect R^2 may be C_{1-8}alkyl for example isopropyl, n-propyl, isobutyl, sec-butyl, n-butyl, tert-butyl, isopentyl, n-pentyl, hexyl, heptyl or octyl; C_{1-8}alkyl interrupted by an oxygen or sulphur atom for example methoxypropyl, ethoxyethyl, propoxymethyl, ethylthioethyl, methylthiopropyl; phenylC_{1-6}alkyl for example benzyl, phenethyl, phenylpropyl or phenylbutyl; C_{3-8}cycloalkyl for example cyclopropyl, cyclobutyl, cyclopentyl

or cyclohexyl; or C_{3-8}cycloalkylC_{1-6}alkyl for example cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.

Preferably R^2 is isobutyl.

There is a chiral centre at -CHR^2- (when R^2 is not hydrogen); it is preferred that this centre has the configuration indicated in formula (II) hereinafter. For most values of R^2 this centre will have the S-stereochemistry under the Cahn-Prelog-Ingold sequence rules.

Particular groups for R^3 include C_{1-6}alkyl for example methyl, ethyl, isopropyl, n-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, isopentyl, n-pentyl or hexyl; C_{1-6}alkyl interrupted by an oxygen or sulphur atom for example methoxyethyl, methoxypropyl, methylthioethyl or 1,1-dimethylmethylthiomethyl ($MeSCMe_2$-); or phenylC_{1-6}alkyl for example benzyl or phenethyl.

Preferably R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl with tert-butyl being most preferred.

The chiral centre at -CHR^3- preferably has the S-configuration indicated in formula (II) hereinafter. For most of R^3 this centre will have the S-stereochemistry.

Particular groups for R^4 include C_{1-6}alkyl for example methyl, ethyl, n-propyl, isopropyl, tert-butyl or n-butyl; C_{1-6}alkyl interrupted by an oxygen or sulphur atom for example hydroxyethyl, methoxyethyl, methylthioethyl or ethoxyethyl; phenylC_{1-6}alkyl for example benzyl, phenethyl or phenylpropyl; or C_{3-8}cycloalkylC_{1-6}alkyl for example cyclopropylmethyl, cyclobutylmethyl or cyclopentylmethyl.

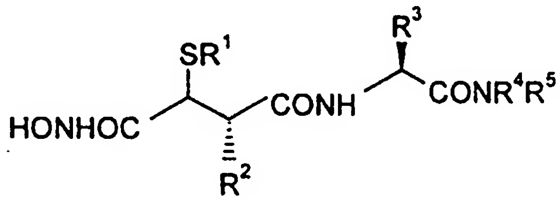
Particularly also, R^4 may be C_{1-6}alkylaminoC_{2-6}alkyl for example methylaminoethyl, di-C_{1-6}alkylaminoC_{2-6}alkyl for example dimethylaminoethyl or heterocyclicC_{1-6}alkyl for example 2-morpholinoethyl, 2-piperidinoethyl, 2-piperazinoethyl or 2-(N-methyl)piperazinoethyl.

Preferably R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl or benzyl. In one aspect R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl. Of these methyl is most preferred.

Particular groups for R^5 are hydrogen and C_{1-6}alkyl for example methyl or ethyl. Preferably R^5 is hydrogen.

In another aspect R^4 and R^5 together with the nitrogen atom to which they are joined form a heterocyclic ring, for example a 5 or 6 membered heterocyclic ring such as morpholino, piperidino, piperazino or N-methylpiperazino. Of these morpholino is preferred.

A particularly suitable class of compounds of the present invention is that of formula (II):



(II)

wherein R^1, R^2, R^3, R^4 and R^5 are as hereinbefore defined.

A preferred class of compounds of the formula (II) is that wherein R^1 is phenyl unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro; C_{1-6}alkylsulphonyl for example methylsulphonyl; trifluoromethyl; C_{1-6}alkyl for example methyl, isopropyl or tert-butyl; C_{1-6}alkoxy for example methoxy; cyano; C_{1-6}alkanoyl for example acetyl; cyanoC_{1-6}alkyl for example 1-cyano-1-methylethyl; or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-OCH_2O-$) group; R^2 is isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4 and R^5 together with the nitrogen atom to which they are joined form a morpholine ring.

In one aspect a preferred class of compounds of the formula (II) is that wherein R^1 is phenyl unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6}alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C_{1-6}alkyl for example methyl, isopropyl or tert-butyl, C_{1-6}alkoxy for example methoxy, cyanoC_{1-6}alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-OCH_2O-$) group; R^2 is isobutyl; R^3 is isobutyl, tert-butyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl; and R^5 is hydrogen.

A further preferred class of compounds of the formula (II) is that wherein R^1 is phenylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6}alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6}alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C_{1-6}alkyl for example methyl, isopropyl or tert-butyl, C_{1-6}alkoxy for example methoxy, cyano, C_{1-6}alkanoyl for example acetyl, cyanoC_{1-6}alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-OCH_2O-$) group; R^2 is isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4 and R^5 together with the nitrogen atom to which they are joined form a morpholine ring.

In another aspect a class of compounds of the formula (II) is that wherein R^1 is phenylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6}alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6}alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C_{1-6}alkyl for example methyl, isopropyl or tert-butyl, C_{1-6}alkoxy for example methoxy, cyanoC_{1-6}alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-OCH_2O-$) group; R^2 is isobutyl; R^3 is isobutyl, tert-butyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl; and R^5 is hydrogen.

In yet another aspect, a preferred class of compounds is that wherein R^1 is quinolinyl, isoquinolinyl, 1-methyl-2-oxodihydroquinolinyl, 1-methyl-2-oxotetrahydroquinolinyl, 2-methyl-1-oxodihydroisoquinolinyl or 2-methyl-1-oxtetrahydroisoquinolinyl; R^2 is isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4.and R^5 together with the nitrogen atom to which they are joined form a morpholine ring.

Suitable pharmaceutically acceptable salts include acid addition salts such as hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulphuric acid. In another aspect suitable salts are base salts such as an alkali metal salt for example sodium or potassium, an alkaline earth metal salt for example calcium or magnesium, or organic amine salt for example triethylamine.

In vivo hydrolysable esters are those pharmaceutically acceptable esters that hydrolyse in the human body to produce the parent compound. Such esters can be identified by administering, for example intravenously to a test animal, the compound under test and subsequently examining the test animal's body fluids. Suitable in vivo hydrolysable esters for carboxy include methoxymethyl and for hydroxy include acetyl.

In order to use a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for the therapeutic treatment (including prophylactic treatment) of mammals including humans, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt or an in vivo hydrolysable ester and pharmaceutically acceptable carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, topical, parenteral, buccal, nasal, vaginal or rectal administration or by inhalation. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions, suspensions, emulsions, creams, ointments, gels, nasal sprays, suppositories, finely divided powders or aerosols for inhalation, and for parenteral use (including intravenous, intramuscular or infusion) sterile aqueous or oily solutions or suspensions or sterile emulsions.

In addition to the compounds of the present invention the pharmaceutical composition of this invention may also contain, or be co-administered (simultaneously or sequentially) with, one or more pharmacological agents of value in treating one or more disease conditions referred to hereinabove.

The pharmaceutical compositions of this invention will normally be administered to humans so that, for example, a daily dose of 0.5 to 75 mg/kg body weight (and preferably of 0.5 to 30 mg/kg body weight) is received. This daily dose may be given in divided doses as necessary, the precise amount of the compound received and the route of administration depending on the weight, age and sex of the patient being treated and on the particular disease condition being treated according to principles known in the art.

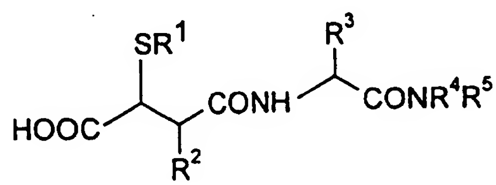
Typically unit dosage forms will contain about 1 mg to 500 mg of a compound of this invention.

Therefore in a further aspect, the present invention provides a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for use in a method of therapeutic treatment of the human or animal body.

In yet a further aspect the present invention provides a method of treating a disease condition mediated by TNF which comprises administering to a warm-blooded animal an effective amount of a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof. The present invention also provides the use of a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof in the preparation of a medicament for use in a disease condition mediated by TNF.

In another aspect the present invention provides a process for preparing a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof which process comprises

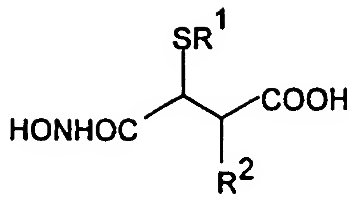
a) reacting a compound of the formula (III):



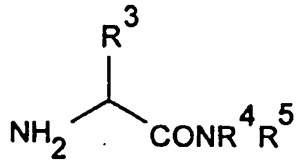
(III)

wherein R^1-R^5 are as hereinbefore defined, or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine or a salt thereof; or

b) coupling a compound of the formula (IV) with a compound of the formula (V):



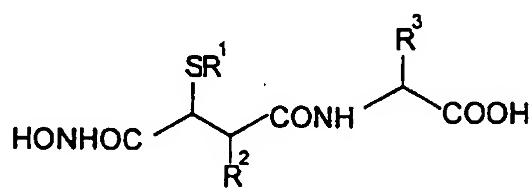
(IV)



(V)

wherein R^1-R^5 are as hereinbefore defined, under standard peptide coupling conditions; or

c) reacting a compound of the formula (VI) with compound of the formula (VII):



(VI)

HNR^4R^5 (VII)

wherein R^1-R^5 are as hereinbefore defined;

wherein any functional group is protected, if necessary, and:

i. removing any protecting groups;

ii. optionally forming a pharmaceutically acceptable salt or in vivo hydrolysable ester.

Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group

with minimum disturbance of groups elsewhere in the molecule.

Specific examples of protecting groups are given below for the sake of convenience, in which "lower" signifies that the group to which it is applied preferably has 1-4 carbon atoms. It will be understood that these examples are not exhaustive. Where specific examples of methods for the removal of protecting groups are given below these are similarly not exhaustive. The use of protecting groups and methods of deprotection not specifically mentioned is of course within the scope of the invention.

A carboxyl protecting group may be the residue of an ester-forming aliphatic or araliphatic alcohol or of an ester-forming silanol (the said alcohol or silanol preferably containing 1-20 carbon atoms).

Examples of carboxy protecting groups include straight or branched chain (1-12C)alkyl groups (eg isopropyl, t-butyl); lower alkoxy lower alkyl groups (eg methoxymethyl, ethoxymethyl, isobutoxymethyl); lower aliphatic acyloxy lower alkyl groups, (eg acetoxymethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl); lower alkoxycarbonyloxy lower alkyl groups (eg 1-methoxycarbonyloxyethyl, 1-ethoxycarbonyloxyethyl); aryl lower alkyl groups (eg benzyl, p-methoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, benzhydryl and phthalidyl); tri(lower alkyl)silyl groups (eg trimethylsilyl and t-butyldimethylsilyl); tri(lower alkyl)silyl lower alkyl groups (eg trimethylsilylethyl); and (2-6C)alkenyl groups (eg allyl and vinylethyl).

Methods particularly appropriate for the removal of carboxyl protecting groups include for example acid-, base-, metal- or enzymically-catalysed hydrolysis.

Examples of hydroxyl protecting groups include lower alkyl groups (eg t-butyl), lower alkenyl groups (eg allyl); lower alkanoyl groups (eg acetyl); lower alkoxycarbonyl groups (eg t-butoxycarbonyl); lower alkenyloxycarbonyl groups (eg allyloxycarbonyl); aryl lower alkoxycarbonyl groups (eg benzoyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl); tri lower alkylsilyl (eg trimethylsilyl, t-butyldimethylsilyl) and aryl lower alkyl (eg benzyl) groups.

Examples of amino protecting groups include formyl, aralkyl groups (eg benzyl and substituted benzyl, p-methoxybenzyl, nitrobenzyl and 2,4-dimethoxybenzyl, and triphenylmethyl); di-p-anisylmethyl and furylmethyl groups; lower alkoxycarbonyl (eg

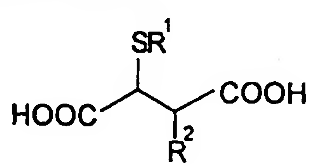
t-butoxycarbonyl); lower alkenyloxycarbonyl (eg allyloxycarbonyl); aryl lower alkoxycarbonyl groups (eg benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl); trialkylsilyl (eg trimethylsilyl and t-butyldimethylsilyl); alkylidene (eg methylidene); benzylidene and substituted benzylidene groups.

Methods appropriate for removal of hydroxy and amino protecting groups include, for example, acid-, base-, metal- or enzymically-catalysed hydrolysis, for groups such as p-nitrobenzyloxycarbonyl, hydrogenation and for groups such as o-nitrobenzyloxycarbonyl, photolytically.

The hydroxylamine group (HONH-), in particular in process variants (b) and (c), is typically O-protected for example with benzyl, 4-methoxybenzyl, 2,4-dimethoxybenzyl, t-butyl or a silyl (for example trimethylsilyl) group.

The compound of the formula (III) may be reacted in the form of the acid or an activated derivative thereof such as an acid halide, acid anhydride or an 'activated' ester such as 1H-benzo[1,2,3]triazol-1-yl, 1-hydroxy-benzo[1,2,3]triazole, pentafluorophenyl or 2,4,5-trichlorophenyl in the presence of a carbodiimide. The reaction of the compound of the formula (III) and hydroxylamine is performed under standard conditions. Typically the reaction of an activated ester of a compound of the formula (III) and hydroxylamine or O-protected hydroxylamine is performed in the presence of a base, for example 2,6-lutidine, (optionally in the presence of dimethylaminopyridine) or N-methylmorpholine in an anhydrous aprotic solvent, for example dimethylformamide, at a non-extreme temperature, for example in the region -30° to +25°, preferably about 0°C.

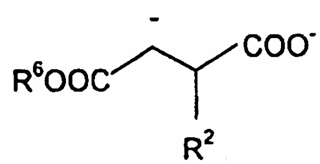
The compound of the formula (III) may be prepared by reacting a compound of the formula (VIII) with a compound of the formula (V):



(VIII)

wherein R^1 and R^2 are as hereinbefore defined, and wherein any functional group is protected and removed as necessary, under standard peptide coupling reaction conditions.

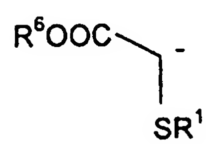
The compounds of the formula (VIII) may be prepared by reacting a source of $-SR^1$ with a dianion (formed in situ) of the formula (IX):



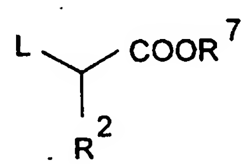
(IX)

wherein R^2 is as hereinbefore defined and R^6 is a protecting group, with subsequent removal of the protecting group if necessary. The dianion may be formed in situ by the action on the corresponding methylene compound, of a strong base, for example lithium di-isopropylamide, in an anhydrous substantially inert solvent, for example tetrahydrofuran, dimethylformamide, dimethylsulfoxide or a mixture thereof, at a low temperature such as -78°C. The corresponding methylene compounds are known in the art, for example see PCT Patent Application WO 94/21625. The source of $-SR^1$ may conveniently be a disulfide R^1-S-S-R^1 which is reacted in situ with the dianion of the formula (IX).

The compounds of the formula (VIII) may also be prepared by reacting a compound of the formula (X) with a compound of the formula (XI):



(X)



(XI)

wherein R^1, R^2 and R^6 are as hereinbefore defined, R^7 is a protecting group and L is a leaving group, for example a methanesulphonyloxy, trifluoromethanesulphonyloxy or p-toluenesulphonyloxy group. The anion of the formula (X) may be formed in situ by the action of a strong base on the corresponding methylene compound.

The compounds of the formulae (IV) and (V) are reacted under standard peptide coupling conditions wherein any functional group is protected as necessary. The compounds of the formula (IV) may be prepared by reacting hydroxylamine with a compound of the formula (VIII), both compounds protected as necessary under conditions similar to those described above for preparing compounds of the formula (III). The compounds of the formula (V) may be prepared under standard conditions for acylation of an amine.

The compounds of the formulae (VI) and (VIII) are reacted under standard conditions for acylation of an amine with any functional groups protected as necessary. The compounds of the formula (VI) may be prepared by methods similar to those described above for preparing compounds of the formula (IV).

The following biological test methods, data and Examples serve to illustrate the present invention.

Isolated Enzyme Assay

The ability of the compounds of this invention to inhibit proTNFα convertase enzyme is assessed in an isolated enzyme assay (termed "CON2"). Partially purified proTNFα convertase enzyme is obtained from the membranes of THP-1 cells as follows. 1.5-2.0x10^6 cells/ml THP-1 cells (initially cultured in RPMI 1640 medium + 10%(v/v) FCS, 10%(v/v) M1, 2mM L-glutamine 100IU/ml penicillin and 100μg/ml streptomycin) are induced in RPMI 1640 containing 1μg/ml LPS (E. coli O111:B4), 2mM Hydroxyurea, 50μg/ml silica and 1%(v/v) FCS at 37°C in a humidified (5%CO_2/95%air) incubator. After 16 hours the cells are harvested from a 5L induction by centrifugation at 640xg for 15 minutes. The cell pellets are washed once in RPMI 1640 without additive (1L per 2x10^{10} cells) and re-centrifuged at 640xg for 10 minutes. Cell pellets are resuspended in 10mM sodium phosphate buffer pH 7.4, containing 1mM $MgCl_2$, 30mM NaCl, 5μM PMSF, 0.02%(w/v) sodium azide (Buffer A) plus a few micrograms DNAase using 3 times the volume of buffer to packed cell pellets. A polytron homogeniser is used to lyse the cells by 5x5sec bursts with 1-2 minutes cooling between each burst. 30 ml of this homogenate is layered onto 10 mls of 41% (w/v) sucrose made in Buffer A and centrifuged at 150,000xg for 1 hour in a swing out rotor. The membrane is collected from the interphase, diluted by addition of 4 volumes Buffer A and centrifuged at 150,000xg for 20 minutes. The pellet is then resuspended in, Buffer A containing 1%(w/v) Triton X-100 to a concentration of 1mg/ml

and mixed for 1 hour at 4°C. The solubilised protein is recovered by centrifugation for 30 minutes at 100,000xg at 4°C. The supernatant is applied to a 25ml gelatin-sepharose 4B column equilibrated in 10mM Tris-HCl pH 8.0, 100mM NaCl, 0.1%(w/v) Triton X-100, 200μM PMSF, 0.02%(w/v) azide, 1μM $ZnCl_2$ (Buffer B). After loading the column is washed with Buffer B. The gelatin-sepharose flowthrough plus the first 10mls of the wash is then recycled overnight (1ml/min) on a 30ml wheatgerm-sepharose column previously equilibrated in 10mM Tris-HCl pH 8.0, 0.1%(w/v) Triton X-100, 200μM PMSF, 0.02%(w/v) azide, 1μM $ZnCl_2$ (Buffer C). After loading the column is washed in Buffer C and the enzyme is eluted with Buffer C containing 300mM N-Acetyl Glucosamine. The active enzyme fractions are applied to a 1ml Mono Q column equilibrated in Buffer C. After loading and washing with Buffer C, enzyme is eluted using a 0-500mM NaCl gradient in Buffer C. Active enzyme fractions are pooled and used as partially purified proTNFα convertase. In all cases the active fractions are assayed using the fluorogenic synthetic peptide substrate assay described below. This enzyme preparation cleaves 21kD soluble proTNFα at the correct cleavage site (Ala-Val) and enzyme activity is inhibited by matrix metalloprotease inhibitors (Gearing, A.J.H. etal., 1995, J Leukocyte Biol., 57,774-777). 4',5'-Dimethoxy-fluoresceinyl Ser.Pro.Leu.Ala.Gln.Ala.Val.Arg.Ser.Ser.Ser.Arg.Cys(4-(3-succinimid-1-yl)-fluorescein)-NH_2 the substrate is used to measure proTNFα convertase enzyme activity in CON2. It was synthesised as follows. The peptidic part of the substrate was assembled on Fmoc-NH-Rink-MBHA-polystyrene resin either manually or on an automated peptide synthesiser by standard methods involving the use of Fmoc-amino acids and O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent with at least a 4- or 5-fold excess of Fmoc-amino acid and HBTU. Ser^1 and Pro^2 were double-coupled. The following side chain protection strategy was employed; $Ser^1(Bu^t)$, Gln^5(Trityl), $Arg^{8,12}$(Pmc or Pbf), $Ser^{9,10,11}$(Trityl), Cys^{13}(Trityl). Following assembly, the N-terminal Fmoc protecting group was removed by treating the Fmoc-peptidyl-resin with piperidine in DMF. The amino-peptidyl-resin so obtained was acylated by treatment for 1.5-2hr at 70°C with 1.5-2 equivalents of 4',5'-dimethoxy-fluorescein-4(5)-carboxylic acid (Khanna & Ullman, Anal Biochem, 108, 156-161, 1980) which had been preactivated with diisopropylcarbodiimide and 1-hydroxybenzotriazole in DMF. The dimethoxyfluoresceinyl-peptide was then simultaneously deprotected and cleaved from the resin by treatment with trifluoroacetic acid

containing 5% each of water and triethylsilane. The dimethoxyfluoresceinyl-peptide was isolated by evaporation, trituration with diethyl ether and filtration. The isolated peptide was reacted with 4-(N-maleimido)-fluorescein in DMF containing diisopropylethylamine, the product purified by RP-HPLC and finally isolated by freeze-drying from aqueous acetic acid. The product was characterised by MALDI-TOF MS and amino acid analysis.

Test compounds are serially diluted in assay buffer (50mM Tris HCl, pH 7.4 containing 0.1% (w/v) Triton X-100 and 2mM $CaCl_2$) and 50μl of each concentration is added to appropriate wells of a 96 well plate and 50μl assay buffer is added to substrate alone (n=6) and substrate +enzyme (n=6) control wells. ProTNFα convertase enzyme (25μl; 0.0144 units/ml in assay buffer) is added to all wells, except substrate alone controls which receive 25μl assay buffer. (NB: One unit of enzyme activity is defined as the convertase enzyme concentration which converts 1nMole substrate/hour).

Plates are incubated for 15 minutes at 26°C, prior to addition of 25μl substrate (40μM stock solution in assay buffer). Plates are then incubated at 26°C for 18 hours and read on a Fluoroskan II fluorometer (plates are also read at time 0 to obtain background values). In this test, generally, compounds are of interest if they have activity below 500nM. By way of example the compound of Example 1 gave a figure of 0.8 nM.

Assessment in human cell line (THF-2)

The ability of the compounds of this invention to inhibit TNFα production is assessed in THP-1 cells which are a human myelomonocytic cell line which synthesise and secrete TNFα when stimulated with lipopolysaccharide. THP-1 cells ($4x10^5$ cells in 160μl medium RPMI 1640 + bicarbonate, penicillin, streptomycin and glutamine) are incubated with 20μl of test compounds (triplicates) in DMSO or appropriate vehicle, in a 96 well tissue culture (TC) plate, for 30 min at 37°C in a humidified (5%CO_2/95%air) incubator, prior to addition of 20μl lipopolysaccharide (LPS) (E. Coli. 0111:B4 (Sigma); final concentration 50 μg/ml). Each assay includes controls of THP-1 cells incubated with medium alone (six wells/plate) or with a standard TNFα inhibitor. The plates are then incubated for 6 hours at 37°C (humidified incubator) after which time 100μl samples are removed from each well and transferred to a 96 well plate for storage at -70°C for subsequent analysis of TNFα concentration by ELISA. In this test, generally, compounds are of interest if they have activity below 10μM.

Assessment in whole blood assay

The ability of the compounds of this invention to inhibit TNFα production is also assessed in a human whole blood assay (HWBA). Human whole blood secretes TNFα when stimulated with LPS. This property of blood forms the basis of an assay which is used as a secondary test for compounds which profile as active in the THP-1 test. Heparinized (10Units/ml) human blood obtained from volunteers is diluted 1:5 with medium (RPMI1640 + bicarbonate, penicillin, streptomycin and glutamine) and incubated (160µl) with 20µl of test compound (triplicates), in DMSO or appropriate vehicle, for 30 min at 37°C in a humidified (5%CO_2/95%air) incubator, prior to addition of 20µl LPS (E. coli. 0111:B4; final concentration 10µg/ml). Each assay includes controls of diluted blood incubated with medium alone (6 wells/plate) or a known TNFα inhibitor as standard. The plates are then incubated for 6 hours at 37°C (humidified incubator), centrifuged (2000rpm for 10 min; 4°C), plasma harvested (50-100µl) and stored in 96 well plates at -70°C before subsequent analysis for TNFα concentration by ELISA. In this test, generally, compounds are of interest if they have activity below 50µM.

In vivo assessment

The ability of the compounds of this invention as *ex vivo* TNFα inhibitors is assessed in the rat. Briefly, groups of male Wistar Alderley Park (AP) rats (180-210g) are dosed with compound (6 rats) or drug vehicle (10 rats) by the appropriate route e.g. peroral (p.o.), intraperitoneal (i.p.) , subcutaneous (s.c.). Ninety minutes later rats are sacrificed using a rising concentration of CO_2 and bled out via the posterior vena cavae into 5 Units of sodium heparin/ml blood. Blood samples are immediately placed on ice and centrifuged at 2000 rpm for 10 min at 4°C and the harvested plasmas frozen at -20°C for subsequent assay of their effect on TNFα production by LPS-stimulated human blood. The rat plasma samples are thawed and 175µl of each sample are added to a set format pattern in a 96U well plate. Fifty µl of heparinized human blood is then added to each well, mixed and the plate is incubated for 30 min at 37°C (humidified incubator). LPS (25µl; final concentration10µg/ml) is added to the wells and incubation continued for a further 5.5 hours. Control wells are incubated with 25µl of medium alone. Plates are then centrifuged for 10 min at 2000 rpm and 200µl of the supernatants are transferred to a 96 well plate and frozen at -20°C for subsequent analysis of TNF concentration by ELISA.

Data analysis by dedicated software calculates for each compound/dose:

$$\text{Percent inhibition of TNF}\alpha = \frac{\text{Mean TNF}\alpha\text{ (Controls) - Mean TNF}\alpha\text{ (Treated)}}{\text{Mean TNF}\alpha\text{ (Controls)}} \times 100$$

Pharmacokinetic test

To evaluate the clearance properties of the compounds of this invention a sensitive ex vivo pharmacokinetic test is employed which utilises the CON2 assay to evaluate clearance rate.

This is a generic test which can be used to estimate the clearance rate of compounds across a range of species. Animals (eg. rats, marmosets) are dosed iv with a soluble formulation of compound and at subsequent time points (e.g. 5, 10, 15, 20, 30, 45, 60, 120 min) blood samples are taken from an appropriate vessel into 10U heparin. Plasma fractions are obtained following centrifugation and the plasma proteins precipitated with ethanol (70% final concentration). After 30 mins at 4°C the plasma proteins are sedimented by centrifugation and the supernatant fraction is evaporated to dryness using a Savant speed vac. The sediment is reconstituted in CON2 assay buffer and subsequently analysed for compound content using the TNF convertase assay (CON2). Briefly, a compound concentration-response curve is constructed for the compound undergoing evaluation. Serial dilutions of the reconstituted plasma extracts are assessed for activity and the amount of compound present in the original plasma sample is calculated using the concentration-response curve taking into account the total plasma dilution factor.

Test as anti-arthritic agent

Activity of a compound as an anti-arthritic is tested as follows. Acid soluble native type II collagen was shown by Trentham et al. [1] to be arthritogenic in rats; it caused polyarthritis when administered in Freunds incomplete adjuvant. This is now known as collagen-induced arthritis (CIA) and similar conditions can be induced in mice and primates. Recent studies have shown that anti-TNF monoclonal antibodies [2] and TNF receptor-IgG fusion proteins [3] ameliorate established CIA indicating that TNF plays a key role in the pathophysiology of CIA. Moreover, the remarkable efficacy reported for anti-TNF monoclonal antibodies in recent rheumatoid arthritis clinical trials indicates that TNF plays a

major role in this chronic inflammatory disease. Thus CIA in DBA/1 mice as described in references 2 and 3 is a tertiary model which can be used to demonstrate the anti-arthritic activity of a compound.

1. Trentham, D.E. et al., (1977) J. Exp. Med., 146, 857.

2. Williams, R.O. et al., (1992) Proc Natl Acad Sci, 89, 9784.

3. Williams, R.O. et al., (1995) Immunology, 84, 433.

In the examples:

(a) NMR spectra were taken at 400 MHz;

(b) DMF means dimethylformamide;

(c) Evaporation of solvents was carried out under reduced pressure;

(d) LDA means lithium di-isopropylamide;

(e) THF means tetrahydrofuran;

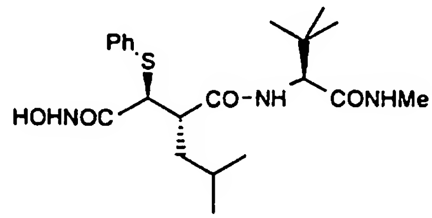
(f) DMSO means dimethylsulphoxide;

(g) AcOH means acetic acid.

(h) DMAP means dimethylaminopyridine.

Example 1

N[2]-[4-(N-Hydroxyamino)-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N[1]-methylamide



N[2]-[4-Hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N[1]-methylamide (2.15 g, 5.1 mmol) was dissolved in DMF (15 ml). 1-Hydroxybenzotriazole (770 mg, 5.7 mmol) was added, followed by N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.09 g, 5.7 mmol). The mixture was stirred at room temperature for one hour. Hydroxylamine hydrochloride (529 mg, 7.6 mmol) was added immediately followed by 2,6-lutidine (830 μl, 7.6 mmol) and DMAP (62 mg, 0.51 mmol). The resulting solution was stirred at room temperature for 3 hours. The resulting mixture was purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 60/40). Elution yielded the title compound (520 mg; yield: 24%): m.p. = 198-200°C; ^{1}H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.84 (d, 3H, J= 6.6 Hz), 0.93 (s, 9H), 0.9-1.0 (m, 1H), 1.3-1.5 (m, 2H), 2.55 (d, 3H, J= 4.8 Hz), 3.00 (m, 1H), 3.56 (d, 1H, J= 11.3 Hz), 4.22 (d, 1H, J= 9.5 Hz), 7.15-7.4 (m, 5H), 7.79 (q, 1H, J= 4.8 Hz), 8.00 (d, 1H, J= 9.5 Hz), 9.0 (s br, 1H), 10.75 (m, 1H); MS (ESI): 446 (M + Na$^+$).

N[2]-[4-Hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N[1]-methylamide used as the starting material was obtained as follows:

(i) To a stirred solution of LDA [32.8 mmol; prepared by addition of 1.6 M n-butyl lithium (20.5 ml, 32.8 mmol) in hexane to a solution of diisopropylamine (4.4 ml, 33.75 mmol) in dry THF (20 ml) at -78°C] cooled at -78°C under argon atmosphere was added 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester [(1)] (3.45 g, 15 mmol) in dry THF (15 ml) dropwise. The mixture was stirred for 90 minutes at -78°C and a solution of diphenyl disulfide (4.2 g, 19 mmol) in dry THF (15 ml) was added. The mixture was stirred for 30 minutes at -78°C, warmed to room temperature and stirred for two hours at room temperature. The solution was

cooled to -78°C and quenched by addition of methanol (6 ml). The solution was warmed to room temperature and the solvents were evaporated in vacuo. Ice was added to the residue and the mixture was acidified with 2N hydrochloric acid to pH 4. The solution was extracted with diethyl ether (3 x 100 ml). The combined organic extracts were dried over $MgSO_4$, filtered and the solvents were removed. The residue was purified by flash chromatography on silica using petroleum ether-ethyl acetate (gradient from 8/2 to 0/10) as eluant to give a mixture of 2S-isobutyl-3(R,S)-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (4.77g; ratio R/S: 50/50). Preparative normal phase HPLC purification using ethyl acetate-cyclohexane (5:95) as eluant gave 2S-isobutyl-3S-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (2.3 g): ^{1}H-NMR ($CDCl_3$): 0.83 (d, 3H, J= 7.3 Hz), 0.85 (d, 3H, J= 7Hz), 1.26 (m, 1H), 1.39 (s, 9H), 1.63 (m, 1H), 1.72 (m, 1H), 2.98 (m, 1H), 3.68 (d, 1H, J= 10.3 Hz), 7.28 (m, 3H), 7.50 (m, 2H).

Further elution gave the other isomer (2S-isobutyl-3R-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester) (2.2 g): ^{1}H-NMR ($CDCl_3$): 0.93 (d, 6H, J= 6.2Hz), 1.35 (s, 9H), 1.6-1.75 (m, 2H), 1.86 (m, 1H), 2.81 (m, 1H), 3.62 (d, 1H, J= 10.3 Hz), 7.30 (m, 3H), 7.49 (m, 2H).

(1) British Biotech Ltd, (Crimmin, M.J.; Beckett, P.R.; Davis, M.H.), Patent WO94/21625 (1994)

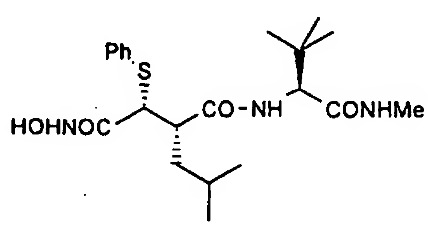
(ii) To 2S-isobutyl-3S-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (2.75 g, 8.1 mmol) in DMF (16 ml) at 0°C was added successively 1-hydroxybenzotriazole (1.32 g, 9.8 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.87 g, 9.8 mmol). After 15 minutes, L-tert-leucine methylamide (1.41 g, 9.8 mmol) was added to the mixture followed by DMAP (195 mg, 1.6 mmol). The mixture was stirred at room temperature for three hours. The mixture was poured into cold water and extracted with diethyl ether (2 x 100 ml). The combined organic layers were washed with saturated sodium bicarbonate, brine and dried over $MgSO_4$. The solvents were evaporated in vacuo and the residue was purified by flash chromatography on silica using ethyl acetate-petroleum ether (3:7) as eluant to give N^2-[2S-isobutyl-3S-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide. (3.0 g, yield: 79%) as a white solid: ^{1}H-NMR ($CDCl_3$): 0.84 (d, 3H, J= 6.6 Hz), 0.90 (d, 3H, J= 6.2 Hz), 1.06 (s, 9H), 1.17 (m, 1H), 1.34 (s, 9H), 1.45 (m, 1H), 1.74 (m, 1H), 2.67 (m, 1H), 2.80 (d,

3H, J= 4.8 Hz), 3.71 (d, 1H, J= 11 Hz), 4.27 (d, 1H, J= 9.2 Hz), 5.95 (s br, 1H), 6.45 (d, 1H, J= 9.2 Hz), 7.27 (m, 3H), 7.47 (m, 2H).

(iii) Trifluoroacetic acid (8.3 ml) was added dropwise to a solution of N^2-[2S-isobutyl-3S-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (3.0 g, 6.05 mmol) in dry dichloromethane (19 ml). The solution was stirred at 0°C overnight. The solvents were evaporated in vacuo. The residue was taken up in toluene and the solvent was removed in vacuo (three times) to give white crystals which were washed with pentane and dried in vacuo to yield N^2-[4-hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide (2.56 g, 97%): ^{1}H-NMR (DMSO d-6): 0.79 (d, 1H, J= 6.6 Hz), 0.87 (d, 1H, J= 6.6 Hz), 0.96 (s, 9H), 1.06 (m, 1H), 1.40 (m, 1H), 1.56 (m, 1H), 2.57 (d, 3H, J= 4.4 Hz), 3.03 (m, 1H), 3.62 (d, 1H, J= 11 Hz), 4.26 (d, 1H, J= 9.2 Hz), 7.43-7.24 (m, 5H), 7.86 (q, 1H, J= 4.4 Hz), 8.13 (d br, 1H, J= 9.2 Hz).

Example 2

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide



N^2-[4-Hydroxy-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide (170 mg, 0.41 mmol) was dissolved in DMF (3.5 ml) and cooled at 0°C. 1-Hydroxybenzotriazole (73 mg, 0.54 mmol) was added, followed by N-methylmorpholine (60 µl, 0.54 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (103 mg, 0.54 mmol). The mixture was stirred at 0°C for one hour. A mixture of hydroxylamine hydrochloride (58 mg, 0.83 mmol) and N-methyl morpholine (91 µl, 0.83 mmol) in DMF (1.5 ml) was added. The resulting solution was stirred at 0°C for one hour then at room temperature overnight. The solvents were evaporated in vacuo and the residue was purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 60/40). Elution yielded the compound of Example 1 (55 mg; yield: 32%). Further elution using the

same solvent gave N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide (49 mg, yield: 29%): m.p.= 194-198°C; ^{1}H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.6 Hz), 0.79 (d, 3H, J= 6.6 Hz), 0.87 (s, 9H), 1.3-1.6 (m, 3H), 2.55 (d, 3H, J= 4.8 Hz), 2.88 (m, 1H), 3.62 (d, 1H, J= 9.5 Hz), 4.12 (d, 1H, J= 9.5 Hz), 7.2-7.5 (m, 5H), 7.69 (d, 1H, J= 9.5 Hz), 7.78 (q, 1H, J= 4.8 Hz), 8.86 (s, 1H), 10.75 (m, 1H); MS (ESI): 446 ($M + Na^+$).

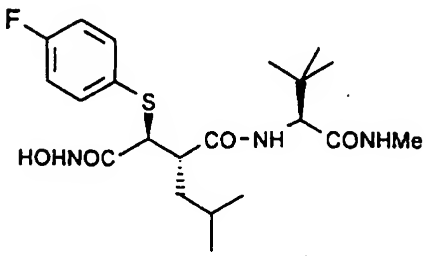
N^2-[4-Hydroxy-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide used as the starting material was obtained as follows:

(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3R-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (478 mg, 1.4 mmol) there was obtained N^2-[2S-isobutyl-3R-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (540 mg, yield: 78%) as a white foam: ^{1}H-NMR ($CDCl_3$): 0.87 (d, 1H, J= 6.6 Hz), 0.88 (d, 1H, J= 6.6 Hz), 0.99 (s, 9H), 1.33 (s, 9H), 1.51 (m, 1H), 1.64 (m, 1H), 1.79 (m, 1H), 2.64 (m, 1H), 2.78 (d, 3H, J= 5.1 Hz), 3.73 (d, 1H, J= 9.9 Hz), 4.14 (d, 1H, J= 9.2 Hz), 5.88 (q, 1H, J= 5.1 Hz), 6.56 (d, 1H, J= 9.2 Hz), 7.30 (m, 3H), 7.49 (m, 2H).

(ii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3R-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (490 mg) there was obtained N^2-[4-hydroxy-2S-isobutyl 3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide (460 mg) as a white solid: ^{1}H-NMR ($CDCl_3$): 0.84 (d, 3H, J= 6.2 Hz), 0.87 (d, 3H, J= 6.2 Hz), 1.06 (s, 9H), 1.55-1.80 (m, 3H), 2.86 (d, 3H, J= 5.1 Hz), 2.97 (m, 1H), 3.84 (d, 1H, J= 6.6 Hz), 4.38 (d, 1H; J= 9.5 Hz), 6.28 (s br, 1H), 7.32 (m, 3H), 7.50 (m, 2H).

Example 3

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N2-[4-hydroxy-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (530 mg, 1.24 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (240 mg, 36%) as a white solid: m.p.= 195-197°C; ¹H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.6 Hz), 0.96 (s, 9H), 1.0 (m, 1H), 1.37 (m, 2H), 2.57 (d, 3H, J= 4.4 Hz), 3.01 (m, 1H), 3.46 (d, 1H, J= 11.4 Hz), 4.26 (d, 1H, J= 9.1 Hz), 7.17 (dd, 2H, J= J'= 8.8 Hz), 7.41 (dd, 2H, J=5.5 Hz, J'= 8.8 Hz), 7.81 (q, 1H, J= 4.4 Hz), 8.03 (d, 1H, J= 9.1 Hz), 8.98 (s br, 1H); 10.55 (s br, 1H); MS (ESI): 464 (M + Na⁺).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.15 g, 5 mmol) and di-(4-fluorophenyl) disulfide(2) (1.4 g), there was obtained 2S-isobutyl-3S-(4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester: ¹H-NMR ($CDCl_3$): 0.90 (d, 1H, J= 6.6 Hz), 0.92 (d, 1H, J= 6.6 Hz), 1.25 (m, 1H), 1.41 (s, 9H), 1.75-1.55 (m, 2H), 2.94 (m, 1H), 3.56 (d, 1H, J= 10.6 Hz), 7.00 (dd, 2H, J=J'= 8.8 Hz), 7.50 (dd, 2H, J= 8.8 Hz, J'= 5.1 Hz) and the other isomer: 2S-isobutyl-3R-(4-fluorophenyl)thio-butan-1,4-dioic acid-4-tert-butyl ester: ¹H-NMR ($CDCl_3$): 0.93 (m, 6H), 1.37 (s, 9H), 1.9-1.3 (m, 3H), 2.77 (m, 1H), 3.54 (d, 1H, J= 10.3 Hz), 7.02 (dd, 2H, J=J'= 8.8 Hz), 7.49 (dd, 2H, J= 8.8 Hz, J'= 5.1 Hz).

(ii) In a manner analogous to that described in Example 1 (ii) except that dichloromethane was used instead of DMF as a solvent, from 2S-isobutyl-3S-(4-fluorophenyl)thiobutan-1,4-dioic

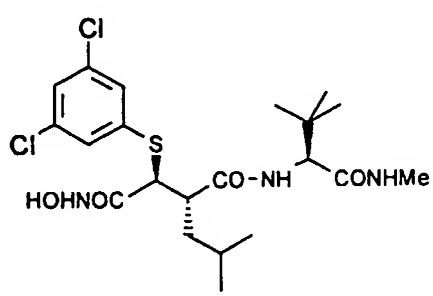
acid-4-tert-butyl ester (800 mg, 2.24 mmol), there was obtained N^2-[2S-isobutyl-3S-(4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (810 mg, 75%) as a white solid: ^{1}H-NMR ($CDCl_3$): 0.84 (d, 3H, J= 6.6 Hz), 0.89 (d, 3H, J= 6.6 Hz), 1.06 (s, 9H), 1.15 (m, 1H), 1.37 (s, 9H), 1.45 (m, 1H), 1.72 (m, 1H), 2.65 (m, 1H), 2.81 (d, 3H, J= 4.6 Hz), 3.61 (d, 1H, J= 11 Hz), 4.27 (d, 1H, J= 9.2 Hz), 5.88 (s br, 1H), 6.44 (d, 1H, J= 9.2 Hz), 6.98 (dd, J=J'= 8.8 Hz), 7.46 (dd, 2H, J= 5.1 Hz, J'= 8.8 Hz).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (800 mg, 1.66 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (700 mg) as a white solid: ^{1}H-NMR (DMSO d-6): 0.78 (d, 1H, J=6.6 Hz), 0.87 (d, 1H, J= 6.6 Hz), 0.97 (s, 9H), 1.05 (m, 1H), 1.6-1.35 (m, 2H), 2.58 (d, 1H, J= 4.4 Hz), 3.03 (m, 1H), 3.54 (d, 1H, J= 11.4 Hz), 4.28 (d, 1H, J= 9.5 Hz), 7.18 (m, 2H), 7.47 (dd, 2H, J= 5.5 Hz, J'= 8.8 Hz), 7.88 (q, 1H, J= 4.4 Hz), 8.13 (d, 1H, J= 9.5 Hz).

(2) Unless stated, the disulfides are prepared from the corresponding commercially available thiols by oxidation with iodine.

Example 4

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (770 mg) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)-

thiosuccinyl]-L-tert-leucine-N^1-methylamide (80 mg, 11%) as a white solid; m.p.= 199-202°C: ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.2 Hz), 0.86 (d, 3H, J= 6.2 Hz), 0.91 (s, 9H), 1.01 (m, 1H), 1.37 (m, 2H), 2.56 (d, 3H, J= 4.4 Hz), 3.10 (m, 1H), 3.68 (d, 1H, J= 11 Hz), 4.24 (d, 1H, J= 9.5Hz), 7.42 (d, 2H, J= 1.8 Hz), 7.46 (t, 1H, J= 1.8 Hz), 7.85 (q, 1H, J= 4.4 Hz), 8.13 (d, 1H, J= 9.5 Hz), 9.1 (s br, 1H), 10.6 (m, 1H); MS (ESI): 518 (M{^{37}Cl,^{37}Cl} + Na$^+$), 516 (M{^{35}Cl,^{37}Cl} + Na$^+$), 514 (M{^{35}Cl,^{35}Cl} + Na$^+$).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-(3,5-dichlorophenyl) disulfide(2) (2.54 g), there was obtained 2S-isobutyl-3S-(3,5-dichlorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.0 g): ^{1}H-NMR ($CDCl_3$): 0.92 (d, 3H, J= 6.6 Hz), 0.94 (d, 3H, J= 6.6 Hz), 1.25 (m, 1H), 1.43 (s, 9H), 1.8-1.5 (m, 2H), 2.97 (m, 1H), 3.70 (d, 1H, J= 10.3 Hz), 7.26 (1H), 7.38 (d, 2H, J= 1.8 Hz) and the other isomer: 2S-isobutyl-3R-(3,5-dichlorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (300 mg): ^{1}H-NMR ($CDCl_3$): 0.94 (m, 6H), 1.40 (s, 9H), 1.8 1.5 (m, 3H), 2.83 (m, 1H), 3.69 (d, 1H, J= 10.2 Hz), 7.28 (1H, t, J= 1.8 Hz), 7.39 (d, 2H, J= 1.8 Hz).

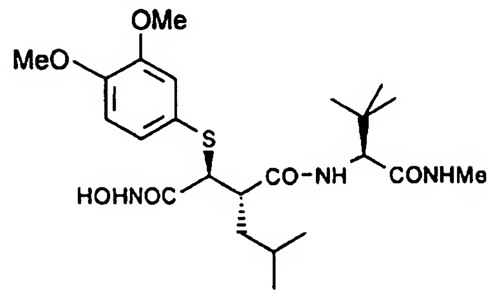
(ii) In a manner analogous to that described in Example 1 (ii) except that dichloromethane was used instead of DMF as a solvent, from 2S-isobutyl-3S-(3,5-dichlorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1 g, 2.45 mmol), there was obtained N^2-[2S-isobutyl-3S-(3,5-dichlorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.0 g, 77%) as a white foam.

MS (ESI): 559 (M{^{37}Cl,^{37}Cl} + Na$^+$), 557 (M{^{35}Cl,^{37}Cl} + Na$^+$), 555 (M{^{35}Cl,^{35}Cl} + Na$^+$)

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(3,5-dichlorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.0 g, 1.9 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (775 mg) as a white solid: ^{1}H-NMR (DMSO d-6): 0.80 (d, 3H, J= 6.6 Hz), 0.87 (d, 3H, J= 6.2 Hz), 0.93 (s, 9H), 1.1 (m, 1H), 1.40 (m, 1H), 1.57 (m, 1H), 2.56 (d, 3H, J= 4.4 Hz), 3.09 (m, 1H), 3.72 (d, 1H, J= 10.6 Hz), 4.26 (d, 1H, J=9.6 Hz), 7.45 (s, 2H), 7.52 (s, 1H), 7.90 (q, 1H, J= 4.4 Hz), 8.21 (d br, 1H, J= 9.6 Hz)

Example 5

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (450 mg) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)-thiosuccinyl]-L-tert-leucine-N^1-methylamide (170 mg) as a white solid: m.p.= 218-220°C; ^{1}H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.98 (s, 9H), 1.0 (m, 1H), 1.40 (m, 2H), 2.58 (d, 3H, J= 4.4 Hz), 2.98 (m, 1H), 3.37 (d, 1H, J= 11.4 Hz), 3.75 (s, 3H), 3.76 (s, 3H), 4.29 (d, 1H, J= 9.5Hz), 6.88 (m, 2H), 6.99 (s, 1H), 7.81 (q, 1H, J= 4.4 Hz), 7.99 (d, 1H, J= 9.5 Hz), 8.98 (s br, 1H); 10.53 (s br, 1H); MS (ESI): 506 (M + Na$^+$).

The starting material was prepared as follows:

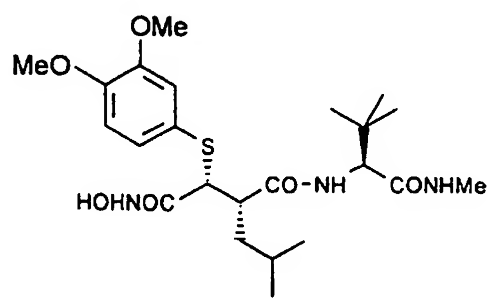
(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.0 g, 4.34 mmol) and di-(3,4-dimethoxyphenyl) disulfide[(2)] (2.0 g), there was obtained 2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.05 g): ^{1}H-NMR ($CDCl_3$): 0.90 (d, 3H, J= 6.6 Hz), 0.91 (d, 3H, J= 6.6 Hz), 1.26 (m, 1H), 1.42 (s, 9H), 1.75-1.55 (m, 2H), 2.96 (m, 1H), 3.54 (d, 1H, J= 10.3 Hz), 3.86 (s, 3H), 3.87 (s, 3H), 6.78 (d, 1H, J= 8.1 Hz), 7.1 (m, 2H) and the other isomer: 2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (550 mg): ^{1}H-NMR ($CDCl_3$): 0.94 (d, 6H, J= 6.6 Hz), 1.38 (s, 9H), 1.75-1.55 (m, 2H), 1.86 (m, 1H), 2.78 (m, 1H), 3.50 (d, 1H, J= 10.3 Hz), 3.87 (s, 3H), 3.88 (s, 3H), 6.80 (d, 1H, J= 8.1 Hz), 7.02 (d, 1H, J= 2.2 Hz), 7.09 (dd, 1H, J= 2.2 Hz, J'= 8.1 Hz).

(ii) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6-lutidine (1 equivalent), from 2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1 g, 2.45 mmol), there was obtained N^2-[2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.1 g, 84%) as a white foam.
MS (ESI): 547 ($M + Na^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (900 mg, 1.7 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-succinyl]-L-tert-leucine-N^1-methylamide (795 mg) as a white solid: MS (ESI): 469 ($M + H^+$), 491($M + Na^+$).

Example 6

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (450 mg) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (170 mg) as a white solid.
^{1}H-NMR (DMSO d-6): 0.79 (d, 3H, J= 6.6 Hz), 0.82 (d, 3H, J= 6.2 Hz), 0.89 (s, 9H), 1.35-1.50 (m, 2H), 1.65 (m, 1H), 2.57 (d, 3H, J= 4.4 Hz), 2.82 (m, 1H), 3.45 (d, 1H, J= 9.9 Hz), 3.77 (s, 3H), 3.79 (s, 3H), 4.13 (d, 1H, J= 9.2Hz), 6.93 (d, 1H, J= 8.4 Hz), 7.04 (m, 2H), 7.73 (d, 1H, J= 9.2 Hz), 7.79 (s br, 1H), 8.86 (s, 1H); 10.65 (s br, 1H); MS (ESI): 506 ($M + Na^+$).

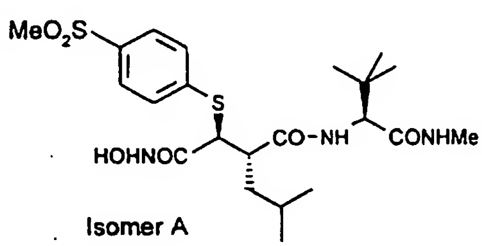
The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6-lutidine (1 equivalent), from 2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (480 mg, 1.2 mmol), there was obtained N^2-[2S-isobutyl-3R-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (555 mg, 88%) as a white foam : MS (ESI): 547 (M + Na^+).

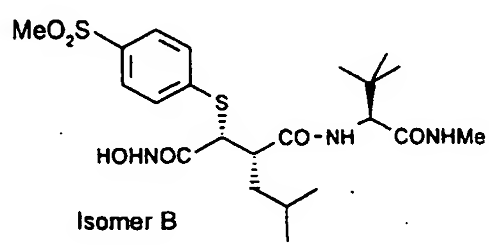
(ii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3R-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (500 mg, 0.95 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thio-succinyl]-L-tert-leucine-N^1-methylamide (256 mg) as a white solid.
MS (ESI): 469 (M + H^+), 491(M + Na^+).

Example 7

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer A) and N^2-[4-(N-Hydroxyamino)-2S-isobutyl-R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer B)



Isomer A



Isomer B

In a manner analogous to that described in the first paragraph of Example 1 except that O-(trimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride, from the mixture of N^2-[4-hydroxy-2S-isobutyl 3(R/S)-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (450 mg, 1:1 mixture) there was obtained N^2-[4-(N-hydroxyamino)

2S-isobutyl-3S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer A) (105 mg) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 45/55): m.p.= 222-224°C: ^{1}H-NMR (DMSO d-6): 0.83 (d, 3H, J= 6.6 Hz), 0.91 (d, 3H, J= 6.2 Hz), 0.94 (s, 9H), 1.08 (m, 1H), 1.44 (m, 1H), 1.55 (m, 1H), 2.60 (d, 3H, J= 4.8 Hz), 3.15 (m, 1H), 3.26 (s, 3H), 3.86 (d, 1H, J= 11.3 Hz), 4.23 (d, 1H, J= 9.2 Hz), 7.61 (d, 2H, J= 8.8 Hz), 7.83 (m, 3H), 8.1 (d, 1H, J= 9.2 Hz), 9.15 (s, 1H), 10.85 (s br, 1H); MS (ESI): 524 (M + Na$^+$).

Further elution with methanol and water/1%AcOH (45:55) gave N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer B) (77 mg) as a white solid: ^{1}H-NMR (DMSO d-6): 0.82 (d, 3H, J= 6.6 Hz), 0.85 (d, 3H, J= 6.6 Hz), 0.93 (s, 9H), 1.48 (m, 3H), 2.62 (d, 3H, J= 4.4 Hz), 3.03 (m, 1H), 3.27 (s, 3H), 3.41 (d, 1H, J= 9.2 Hz), 4.21 (d, 1H, J= 9.2 Hz), 7.71 (d, 2H, J= 8.4 Hz), 7.89 (m, 4H), 8.97 (s, 1H), 10.9 (s br, 1H); MS (ESI): 524 (M + Na$^-$).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) except that di-(4-(methylsulfonyl)phenyl) disulfide was dissolved in DMF instead of THF, from 2R-isobutyl-butan-1,4-dioic acid-4-tert-butyl ester (1.31 g) and di-(4-(methylsulfonyl)phenyl) disulfide[(3)] (2.15 g), there was obtained a mixture of 2S-isobutyl-3(R/S)-(4-(methylsulfonyl)phenyl)thio-butan-1,4-dioic acid-4-tert-butyl ester (800 mg, 1:1 mixture): ^{1}H-NMR ($CDCl_3$): 0.94 (m, 6H), 1.38 (s, 9H, isomer B), 1.42 (s, 9H, isomer A), 1.8-1.3 (m, 3H), 2.89 (m, 1H, isomer B), 3.01 (m, 1H, isomer A), 3.05 (s, 3H), 3.85 (d, 1H, J= 10.2 Hz), 7.63 (m, 2H), 7.86 (m, 2H).

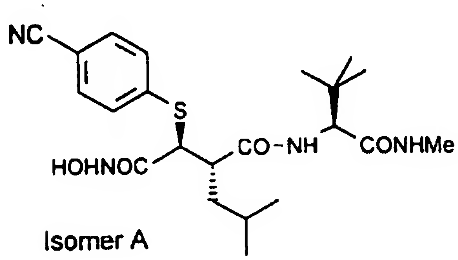
(ii) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6 lutidine (1 equivalent), from the mixture of 2S-isobutyl-3(R/S)-(4-(methylsulfonyl)phenyl)thio-butan-1,4-dioic acid-4-tert-butyl ester (800 mg, 1:1 mixture), there was obtained a mixture of N^2-[2S-isobutyl 3(R/S)-(4-(methylsulfonyl)phenyl)thio 4-tert-butyloxy succinyl]-L-tert-leucine-N^1-methylamide (800 mg, 78%) as a white foam. MS (ESI): 565 (M + Na$^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from the mixture of N^2-[2S-isobutyl 3(R/S)-4-(methylsulfonyl)phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (800 mg, 1.4 mmol, 1:1 mixture) there was obtained a mixture of N^2-[4-hydroxy-2S-isobutyl-3(R/S)-4-(methylsulfonyl)phenylthio succinyl]-L-tert-leucine-N^1-methylamide (500 mg) as a white solid: MS (ESI): 509 (M + Na^+).

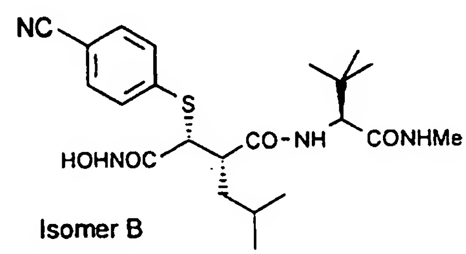
(3) Bordwell, F.G. et al.; J. Am. Chem. Soc., 1953, 75, 6019

Example 8

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer A) and N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer B)



Isomer A



Isomer B

In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (300 mg, 0.7 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-cyanophenyl)-thiosuccinyl]-L-tert-leucine-N^1-methylamide (68 mg, 22%) as a white solid (isomer A): m.p.= 238-241°C: ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.88 (s, 3H); 0.97-1.08 (m, 1H), 1.3-1.53 (m, 2H), 2.55 (d, 3H, J= 4.8 Hz), 3.06-3.15 (m, 1H), 3.80 (d, 1H, J= 11 Hz), 4.17 (d, 1H, J= 9.2 Hz), 7.51 (d, 2H, J= 8.8 Hz), 7.73 (d, 2H, J= 8.5 Hz), 7.75-7.83 (m, 1H), 8.09 (d, 1H, J = 9.2 Hz), 9.05-9.15 (m,1H), 10.75-10.90 (m, 1H) and N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (20 mg) as a white solid (isomer B) formed by epimerisation during the reaction: ^{1}H-NMR

(DMSO d-6): 0.76 (d, 3H, J= 6 Hz), 0.80 (d, 3H, J = 6 Hz), 0.88 (s, 9H), 1.38-1.6 (m, 3H), 2.56 (d, 3H, J= 4.4 Hz), 2.97-3.04 (m, 1H), 3.88 (d, 1H, J= 9.5 Hz), 4.15 (d, 1H, J= 9.5 Hz), 7.60 (d, 2H, J= 8 Hz), 7.80 (d, 2H, J= 8.4 Hz), 7.75-7.85 (m, 2H), 8.94 (s, 1H), 10.85 (s, 1H).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-(4-cyanophenyl) disulfide[(4)] (1.8 g), there was obtained 2S-isobutyl-3S-(4-cyanophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.2 g): ^{1}H-NMR ($CDCl_3$): 0.92 (m, 6H), 1.3 (m, 1H), 1.41(s, 9H), 1.8-1.55 (m, 2H), 2.99 (m, 1H), 3.82 (d, 1H, J= 9.9 Hz), 7.56 (m, 4H) and the other isomer: 2S-isobutyl-3R-(4-cyanophenyl)-thiobutan-1,4-dioic acid-4-tert-butyl ester (300 mg): ^{1}H-NMR ($CDCl_3$): 0.92 (d, 6H, J= 5.9 Hz), 1.37 (s, 9H), 1.8-1.5 (m, 3H), 2.89 (m, 1H), 3.82 (d, 1H, J= 10.2 Hz), 7.57 (m, 4H).

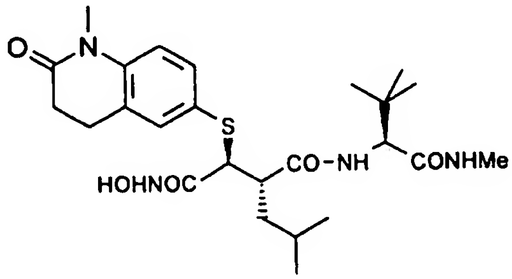
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(4-cyanophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.1 g, 3 mmol), there was obtained N^2-[2S-isobutyl-3S-(4-cyanophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.09 g, 74%) as a white foam: MS (ESI): 512 ($M + Na^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(4-cyanophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.09 g, 2.2 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (737 mg) as a white solid: MS (ESI): 434 ($M + H^+$), 456 ($M + Na^+$).

[(4)] Krishnamurthy, S. and al.; J. Org. Chem., 1989, 54, 4458

Example 9

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (360 mg, 0.73 mol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (75 mg, 20%) as a white solid: m.p.= 235°C (decomposition): ^{1}H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.97 (s, 9H), 0.95-1.05 (m, 1H), 1.3-1.5 (m, 2H), 2.57 (d, 3H, J= 4.8 Hz), 2.5-2.6 (m, 2H), 2.83 (m, 2H), 3.00 (m, 1H), 3.24 (s, 3H), 3.46 (d, 1H, J= 11.3 Hz), 4.26 (d, 1H, J= 9.2 Hz), 7.02 (d, 1H, J= 8.5 Hz), 7.2 (s, 1H); 7.26 (d, 1H, J= 8.5 Hz), 7.8-7.85 (m, 1H), 8.00 (d, 1H, J= 9 Hz), 8.9-9.1 (m, 1H); 10.5-10.8 (m, 1H); MS (ESI): 529 (M + Na$^+$).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.3 g, 5.65 mmol) and di-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) disulfide[(5)] (2.8 g), there was obtained 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (950 mg) after purification on C18 preparative HPLC eluting with aqueous ammonium carbonate (0.2%)-acetonitrile (gradient from 100:0 to 72.5:27.5): MS (ESI): 444 (M + Na$^+$).

Further elution gave the other isomer: 2S-isobutyl-3R-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (603 mg): MS (ESI): 444 (M + Na$^+$).

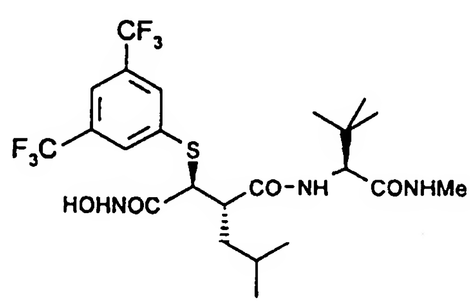
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl 3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (390 mg, 0.92 mmol), there was obtained N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (250 mg,) as a white foam after C18 preparative HPLC eluting with aqueous ammonium carbonate (0.2%)-acetonitrile (gradient from 90:10 to 50:50): MS (ESI): 570 (M + Na$^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (410 mg, 0.75 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (370 mg) as a white solid: ^{1}H-NMR (CDCl$_3$): 0.84 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.3 Hz), 1.03 (s, 9H), 1.34 (m, 1H), 1.46 (m, 1H), 1.70 (m, 1H), 2.63 (m , 2H), 2.82 (d, 3H, J= 4.7 Hz), 2.86 (m, 2H), 2.93 (m, 1H), 3.81 (d, 1H, J= 9.2 Hz), 4.37 (d, 1H, J= 9.6 Hz), 6.38 (d, 1H, J= 8.4 Hz), 7.29 (d, 1H, J= 2.2 Hz), 7.40 (dd, 1H, J= 8.4 Hz, J'= 2.2 Hz), 7.7 (s br, 1H).

[5] Imperial Chemical Industries PLC, ICI-Pharma S.A.; (Bruneau, P.); EPA-462812

Example 10

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N^2-[4-hydroxy-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (500 mg, 0.91 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl 3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (42 mg) as a white solid: m.p.= 225-230°C; ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (s br, 12H), 1.0-1.1 (m, 1H), 1.3-1.5 (m, 2H), 2.56 (d, 3H, J= 4.8 Hz), 3.1-3.2 (m, 1H), 3.77 (d, 1H, J= 11.3 Hz), 4.25 (d, 1H, J= 9.5 Hz), 7.85-7.90 (m, 1H,), 7.94 (s, 1H), 8.04 (s, 2H), 8.15-8.21 (m, 1H), 9.0-9.12 (m, 1H), 10.8-10.92 (m, 1H); MS (ESI): 582 (M + Na^+).

The starting material was prepared as follows:

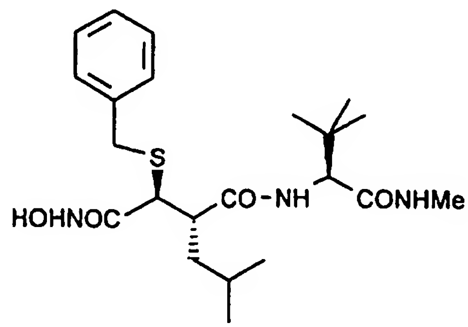
(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-[3,5-di-(trifluoromethyl)phenyl] disulfide[(2)] (3.66 g), there was obtained 2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3 g) after purification on C18 preparative HPLC eluting with methanol and water/1%AcOH (gradient from 20/80 to 70/30): MS (ESI): 497 (M + Na^+); the other isomer 2S-isobutyl-3R-(3,5-di-trifluoromethyl)phenylthiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3 g, 2.7 mmol), there was obtained N^2-[2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.2 g) as a white foam: MS (ESI): 623 (M + Na^+).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.1g, 1.8 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(3,5-trifluoromethyl)phenyl)thio succinyl]-L-tert-leucine-N^1-methylamide (990 mg) as a white solid : MS (ESI): 545 (M + H^+), 567 (M + Na^+).

Example 11

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyldimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride and no base was added, from N^2-[4-hydroxy-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N^1-methylamide (150 mg, 0.35 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N^1-methylamide (78 mg) as a white solid after addition of 1 ml of 1N hydrochloric acid to the crude mixture at the end of the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40) m.p.= 200-205°C; ^{1}H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.95 (s, 9H), 1.00 (m, 1H), 1.45-1.30 (m, 2H), 2.55 (d, 3H, J= 4.4 Hz), 3.10 (m, 1H), 3.20 (d, 1H, J= 15 Hz), 3.83 (d, 1H, J= 12 Hz), 4.04 (d, 1H, J= 12 Hz), 4.26 (d, 1H, J= 9.5 Hz), 7.30-7.20 (m, 5H), 7.85 (d, 1H, J= 4.4 Hz), 8.05 (d, 1H, J= 9.5 Hz), 9.0 (s br, 1H), 10.64 (s br , 1H); MS (ESI): 460 (M + Na^+).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and dibenzyl disulfide (3.66 g), there was obtained 2S-isobutyl-3R-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (1.48 g): ^{1}H-NMR ($CDCl_3$): 0.82 (d, 3H, J=6 Hz), 0.85 (d, 3H, J=5.8 Hz); 1.35 (m, 1H), 1.65-1.50 (m, 2H), 2.70 (m, 1H), 3.10 (d, 1H, J= 10 Hz), 3.84(d, 1H, J= 11.7 Hz), 3.87 (d, 1H, J= 11.7 Hz), 7.35-7.20 (m, 5H).

(ii) To a stirred solution of LDA [9.24 mmol; prepared by addition of 2.5 M n-butyl lithium (3.69 ml, 9.24 mmol) in hexane to a solution of diisopropylamine (1.29 ml, 9.24 mmol) in dry THF (5 ml) at -78°C] cooled at -78°C under an argon atmosphere was added 2S-isobutyl-3R-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (1.48 g, 4.20 mmol) in dry THF (10 ml) dropwise. The mixture was stirred for 15 minutes at -78°C, warmed to room temperature and stirred for two hours at room temperature. The solution was cooled to -78°C and quenched at -78°C by addition of methanol (3 ml). The solution was warmed to room temperature and the solvents were evaporated in vacuo. The residue was dissolved in dichloromethane and was washed successively with 1N hydrochloric acid and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed. The residue was purified by C18 preparative HPLC using methanol-aqueous 0.2% ammonium carbonate (60:40) as eluant. The fractions were collected, acidified to pH 2 with 2N hydrochloric acid and extracted with ether (2x100 ml). The organic layer was dried over $MgSO_4$ and evaporated to give 2S-isobutyl-3S-benzyl-thiobutan-1,4-dioic acid-4-tert-butyl ester (250 mg) as an oil: ^{1}H-NMR ($CDCl_3$): 0.84 (d, 3H, J= 6 Hz), 0.86 (d, 3H, J= 6 Hz), 1.20 (m, 1H), 1.50 (s, 9H), 1.65 (m, 2H), 2.92 (m, 1H), 3.16 (d, 1H, J= 10 Hz), 3.85 (s, 2H), 7.35-7.20 (m, 5H).

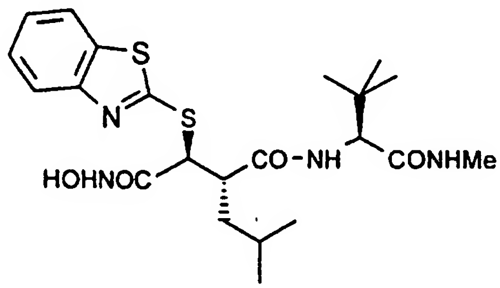
(iii) In a manner analogous to that described in Example 1 (ii) except that 2,6-lutidine (1 equivalent) was used instead of DMAP, from 2S-isobutyl-3S-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (225 mg, 0.64 mmol), there was obtained N^2-[2S-isobutyl-3S-benzylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (254 mg, 83 %) as a white foam.

MS (ESI): 501 (M + Na^+).

(iv) In a manner analogous to that described in example 1 (iii), from N^2-[2S-isobutyl-3S-benzylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (250 mg, 0.52 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N^1-methyl-amide (165 mg) as a white solid: MS (ESI): 423 (M + H^+), 445 (M + Na^+).

Example 12

N^2-[4-(N-Hydroxyamino)-2S-isobutyl 3S-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1 except that O-(tert-butyldimethylsilyl)hydroxylamine (1.5 equivalents) was used instead of hydroxylamine hydrochloride and 1.3 equivalents of 2,6-lutidine was used, from N^2-[4-hydroxy-2S-isobutyl 3(R/S)-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (600 mg, 1.29 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (125 mg) as a white solid after addition of 2 ml of 3N hydrochloric acid to the crude mixture at the end of the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40): ^{1}H-NMR (DMSO d-6): 0.81 (d, 3H, J= 6.6 Hz), 0.87 (d, 3H, J= 6.6 Hz), 0.92 (s, 9H), 1.09 (m, 1H), 1.39 (m, 1H), 1.59 (m, 1H), 2.56 (d, 3H, J= 4.4 Hz), 3.20 (m, 1H), 4.17 (d, 1H, J= 9.9 Hz), 4.35 (d, 1H, J= 11 Hz), 7.39 (t, 1H, J= 7.7 Hz), 7.50 (t, 1H, J= 7.7 Hz), 7.81 (m, 1H), 7.89 (d, 1H, J= 7.7 Hz), 8.01 (d, 1H, J= 7.7 Hz), 8.09 (d, 1H, J=9.2Hz), 9.13 (s, 1H), 10.95 (s, 1H); MS (ESI): 481 (M + H$^+$), 503 (M + Na$^+$). Further elution gave N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) except that the solution of the dianion in THF cooled to -78°C was transferred through a double-ended needle into a slurry of di-(benzothiazol-2-yl) disulfide[(2)] in DMSO-THF (20 ml: 10 ml) cooled at 0°C and the mixture was stirred at 0°C for one hour, from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.15g,

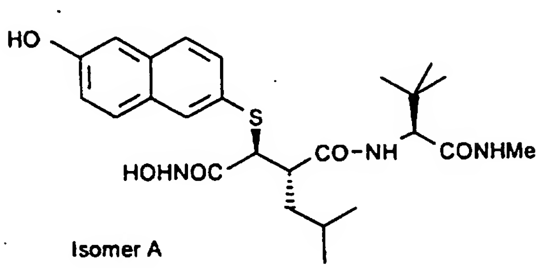
5 mmol) and di-(benzothiazol-2-yl) disulfide(2) (1.4 g), there was obtained a mixture of 2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (830 mg, 1:1 mixture) after purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (70/30): ^{1}H-NMR ($CDCl_3$): 0.96-0.87 (m, 6H), 1.41 and 1.42 (s, 9H), 1.9-1.6 (m, 3H), 3.20 and 3.29 (m, 1H), 4.77 (d, 1H, J= 8 Hz) and 4.79 (d, 1H, J= 7.6 Hz), 7.34 (m, 1H), 7.44 (m, 1H), 7.77 (m, 1H), 7.89 (m, 1H)

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (800 mg, 2.0 mmol), there was obtained N^2-[2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (983 mg, 94%) as a white foam: MS (ESI): 522 (M + H^+), 544 (M + Na^+), 560 (M + K^+).

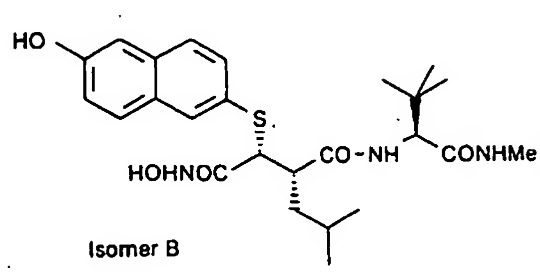
(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (900 mg, 1.72 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (725 mg, 91%) as a white solid: MS (ESI): 466 (M + H^+), 488 (M + Na^+).

Example 13

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer A) and N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer B)



Isomer A



Isomer B

In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride and no base was added, from N^2-[4-hydroxy-2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (650 mg, 1.26 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 10 drops) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/ 1% AcOH (gradient from 30/70 to 60/40) N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer A) (65 mg, 10%) as a white solid: ^{1}H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.84 (d, 3H, J= 6.6 Hz), 0.95 (s, 9H), 1.0 (m, 1H), 1.35-1.55 (m, 2H), 2.55 (d, 3H, J= 4.7 Hz), 3.05 (m, 1H), 3.56 (d, 1H, J= 11.4 Hz), 4.26 (d, 1H, J= 9.1 Hz), 7.1 (m, 2H), 7.36 (d, 1H, J= 8.4 Hz), 7.61 (d, 1H, J= 8.8 Hz), 7.68 (d, 1H J= 9.5 Hz), 7.77 (s, 1H), 7.8 (s br, 1H), 8.03 (d, 1H, J= 9.1 Hz), 8.93 (s br, 1H), 9.81 (s, 1H), 10.58 (s br, 1H); MS (ESI): 512 (M + Na^+).

Further elution with methanol and water/ 1% AcOH (60/40) gave N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (Isomer B) (30 mg, 5%) as a white solid formed by epimerisation during the reaction: ^{1}H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.6 Hz), 0.80 (d, 3H, J= 6.6 Hz), 0.86 (s, 9H), 1.4 (m, 1H), 1.5 (m, 1H), 1.65 (m, 1H), 2.55 (d, 3H, J= 4.7 Hz), 2.88 (m, 1H), 3.61 (d, 1H, J= 9.5 Hz), 4.12 (d, 1H, J= 9.1 Hz), 7.1 (m, 2H), 7.45 (d, 1H, J= 8.8 Hz), 7.67 (d, 1H, J= 8.8 Hz), 7.74 (d, 1H, J= 9.9 Hz), 7.75 (m, 1H), 7.78 (m, 1H), 7.9 (s br, 1H), 8.85 (s br, 1H), 9.86 (s, 1H), 10.8 (s br, 1H); MS (ESI): 490 (M + H^+), 512 (M + Na^+).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (1.92 g, 8.4 mmol) and di-(6-acetoxynaphth-2-yl) disulfide[(6)] (4.0 g), there was obtained 2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.52g, 41%): ^{1}H-NMR ($CDCl_3$): 0.93 (d, 3H, J= 6.2 Hz), 0.94 (d, 3H, J= 6.2 Hz), 1.33 (s, 9H), 1.62-1.7 (m, 1H), 1.7-1.76 (m, 1H), 1.86-1.93 (m, 1H), 2.35 (s, 3H), 2.85 (m, 1H), 3.72 (d, 1H, J= 10.2 Hz), 7.23-7.26 (m, 1H), 7.53-7.56 (m, 2H), 7.73 (d, 1H, J= 8.4 Hz), 7.78 (d, 1H, J= 8.8 Hz), 7.99 (s br, 1H); MS (ESI): 469 (M + Na^+); the other isomer 2S-isobutyl-3R-(6-

acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

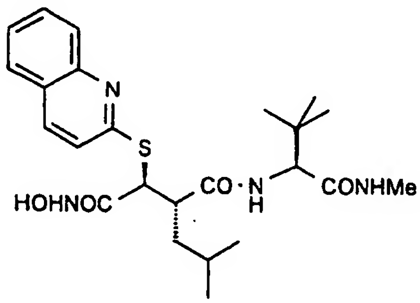
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.5 g, 3.36 mmol), there was obtained N^2-[2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.26 g, 65%) as an off-white solid: ^{1}H-NMR ($CDCl_3$): 0.87 (d, 3H, J= 6.6 Hz), 0.89 (d, 3H, J= 6.6 Hz), 0.98 (s, 9H), 1.30 (s, 9H), 1.53 (m, 1H), 1.65 (m, 1H), 1.86 (m, 1H), 2.35 (s, 3H), 2.7 (m, 1H), 2.76 (d, 3H, J= 4.4 Hz), 3.79 (d, 1H, J= 10.2 Hz), 4.28 (d, 1H, J= 9.5 Hz), 6.58-6.66 (m, 2H), 7.25 (m, 1H), 7.53-7.57 (m, 2H), 7.73 (d, 1H, J= 8.4 Hz), 7.77 (d, 1H, J= 9.1 Hz), 7.99 (s, 1H); MS (ESI): 573 ($M + H^+$), 595 ($M + Na^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (780 mg, 1.36 mmol) there was obtained after purification by flash chromatography on silica using ethyl acetate-methanol (4:1) as eluant N^2-[4-hydroxy-2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio succinyl]-L-tert-leucine-N^1-methylamide (650 mg, 92%) as a white solid: ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 5.8 Hz), 0.82 (d, 3H, J= 5.5 Hz), 0.92 (s, 9H), 1.55-1.65 (m, 3H), 2.32 (s, 3H), 2.62 (d, 3H, J= 4.4 Hz), 2.8 (m, 1H), 3.88 (d, 1H, J= 7.3 Hz), 4.03 (m, 1H), 7.3 (dd, 1H, J= 2.2 Hz, J'= 8.9 Hz), 7.56 (dd, 1H, J= 1.8 Hz, J'= 8.8 Hz), 7.63 (d, 1H, J= 2 Hz), 7.78 (m, 1H), 7.8-7.88 (m, 3H), 7.98 (s, 1H); MS (ESI): 539 ($M + Na^+$), 555 ($M + K^+$).

[(6)]Prepared by acetylation using the standard method (acetyl chloride, triethylamine in THF) from the corresponding commercially available disulfide.

Example 14

N[2]-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N[1]-methylamide



In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyldimethylsilyl)hydroxylamine (1.3 equivalent) was used instead of hydroxylamine hydrochloride, 2,6-lutidine (1.3 equivalent) and DMAP (0.1 equivalent) were used as a base, from N[2]-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N[1]-methylamide (400 mg, 0.87 mmol) there was obtained N[2]-[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N[1]-methylamide (135 mg) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40): m.p.= 188-190°C; ^{1}H-NMR (DMSO d-6): 0.82 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.6 Hz), 0.92 (s, 9H), 1.16 (m, 1H), 1.42 (m, 1H), 1.62 (m, 1H), 2.55 (d, 3H, J= 4.4 Hz), 3.19 (m, 1H), 4.14 (d, 1H, J= 9.2 Hz), 4.54 (d, 1H, J= 11.4 Hz), 7.30 (d, 1H, J= 8.8 Hz), 7.52 (t, 1H, J= 7.3 Hz), 7.76 (m, 2H), 7.90 (d, 1H, J= 8.8 Hz), 8.16-8.04 (m, 3H), 9.05 (s br, 1H), 10.75 (s br, 1H); MS (ESI): 475 $(M + H^+)$, 497 $(M + Na^+)$.

Further elution gave N[2]-[4-(N-hydroxyamino)-2S-isobutyl-3R-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N[1]-methylamide contaminated with minor impurities.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.1 g, 4.8 mmol) and di-(quinolin-2-yl) disulfide[(2)] (1.4 g), there was

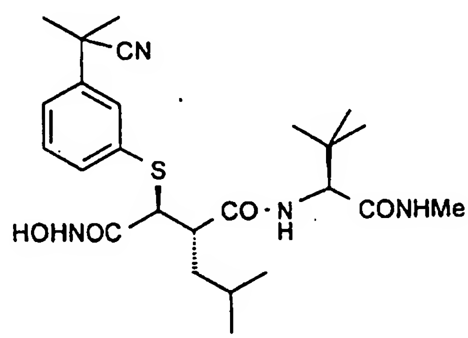
obtained 2S-isobutyl-3(R/S)-(quinolin-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.14 g, 68 %): MS (ESI): 412 (M + Na$^+$).

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(quinolin-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.08 g, 2.8 mmol), there was obtained N^2-[2S-isobutyl-3(R/S)-(quinolin-2-yl)thio-4-tert-butyloxy-succinyl]-L-tert-leucine-N^1-methylamide (1.16 g, 80%, 4:6 mixture) as a white foam.

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3(R/S)-(quinolin-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (800 mg, 1.55 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (410 mg) as a white solid: MS (ESI): 482 (M + Na$^+$).

<u>Example 15</u>

<u>N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N^1-methylamide</u>



In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1 equivalent) was used as a base, from N^2-[4-hydroxy-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N^1-methylamide (410 mg, 0.86 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N^1-methylamide (170 mg, 40%) as a white solid after addition of 2 ml of 2N hydrochloric acid to the crude mixture at the end of

the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 65/35): ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.94 (s, 9H), 1.01 (m, 1H), 1.37 (m, 1H), 1.47 (m, 1H), 1.69 (s, 6H), 2.57 (d, 3H, J= 4.4 Hz), 3.04 (m, 1H), 3.62 (d, 1H, J= 11.4 Hz), 4.25 (d, 1H, J= 9.2 Hz), 7.44-7.35 (m, 4H), 7.81 (m, 1H), 8.04 (d, 1H, J= 9.2 Hz), 9.05 (s, 1H), 10.72 (s, 1H); MS (ESI): 513 (M + Na$^+$).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (0.91 g, 3.9 mmol) and di-[3-(1-cyano-1-methylethyl)phenyl] disulfide[(2)] (1.6 g, 4.5 mmol), there was obtained 2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)-phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester (565 mg, 36 %): MS (ESI): 428 (M + Na$^+$). Further elution gave 2S-isobutyl-3R-[3-(1-cyano-1-methylethyl)phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester.

(ii) In a manner analogous to that described in Example 1 (ii) except that no DMAP was added, from 2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester (565 mg, 1.4 mmol), there was obtained N^2-[2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (495 mg, 67%) as a white foam: MS (ESI): 554 (M + Na$^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (492 mg, 0.93 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N^1-methylamide (440 mg, 99%) as a white solid: MS (ESI): 498 (M + Na$^+$).

(iv) Di-[3-(1-cyano-1-methylethyl)phenyl] disulfide was prepared as follows:

1) alkylation of (3-bromophenyl)acetonitrile with excess sodium hydride/methyl iodide in THF gave 2-(3-bromophenyl) 2-methylpropionitrile (yield: 100%)

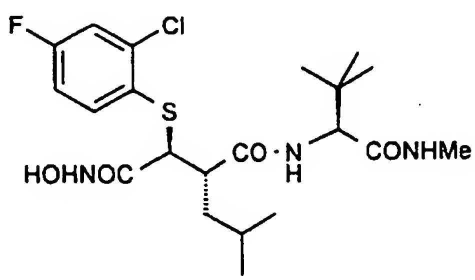
2) 2-(3-(tert-butylthio)phenyl) 2-methylpropionitrile was obtained from 2-(3-bromophenyl) 2-methylpropionitrile by reaction with tert-butyl mercaptan (1.3 eq.), potassium tert-butoxide(1.35 eq.) in DMSO at 80°C in the presence of $Pd(PPh_3)_4$ (0.05 eq) (yield: 37%)

3) 2-(3-mercaptophenyl) 2-methylpropionitrile was obtained from 2-(3-(tert-butylthio)phenyl) 2-methylpropionitrile by reaction with trifluoroacetic acid (1.7 eq.) and trifluoromethylsulfonic acid (1 eq.) in thioanisole at room temperature (yield 82%)

4) oxidation of 2-(3-mercaptophenyl) 2-methyl propionitrile in DMSO gave di-[3-(1-cyano-1-methylethyl)phenyl] disulfide (yield: 73%)

Example 16

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalent) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1 equivalent) was used as a base, from N^2-[4-hydroxy-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (1 g, 2.1 mmol) there was obtained N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluoro-phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (451 mg, 45%) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 65/35): ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.92 (s, 9H), 1.03 (m, 1H), 1.37 (m, 1H), 1.49 (m, 1H), 2.56 (d, 3H, J= 4.8 Hz), 3.69 (d, 1H, J= 11 Hz), 4.20 (d, 1H, J= 9.5 Hz), 7.23 (td, 1H, J_t= 8.8 Hz, J_d= 2.9 Hz), 7.46 (dd, 1H, J= 2.9 Hz, J'= 8.8 Hz), 7.80 (dd, 1H, J= 4 Hz, J'= 8.8 Hz), 9.03 (s, 1H), 10.72 (s, 1H); MS (ESI): 498 (M ^{35}Cl + Na^+), 500 (M ^{37}Cl + Na^+).

The starting material was prepared as follows:

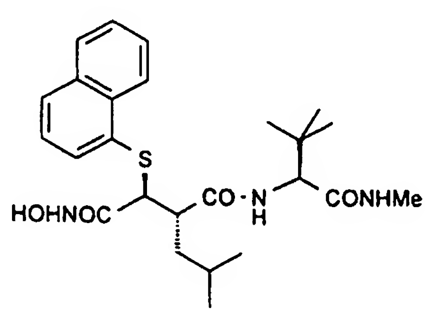
(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (2.7 g, 11.8 mmol) and di-[2-chloro-4-fluorophenyl] disulfide(2) (4.2 g, 13 mmol), there was obtained 2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (2.5 g, 54 %): MS (ESI): 413 (M ^{35}Cl + Na^{+}), 415 (M ^{37}Cl + Na^{+}). Further elution gave 2S-isobutyl-3R-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester.

(ii) In a manner analogous to that described in Example 1 (ii) except that no DMAP was added, from 2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (2.5 g, 6.4 mmol), there was obtained N^{2}-[2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio 4-tert-butyloxysuccinyl]-L-tert-leucine-N^{1}-methylamide (2.38 g, 72%) as a white foam. MS (ESI): 539 (M ^{35}Cl + Na^{+}), 541 (M ^{37}Cl + Na^{+}).

(iii) In a manner analogous to that described in Example 1 (iii), from N^{2}-[2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^{1}-methylamide (2.3 g, 4.4 mmol) there was obtained N^{2}-[4-hydroxy-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio succinyl]-L-tert-leucine-N^{1}-methylamide (2.0 g, 97%) as a white solid: MS (ESI): 483 (M ^{35}Cl + Na^{+}), 485 (M ^{37}Cl + Na^{+}).

Example 17

N^{2}-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N^{1}-methylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.3 equivalents) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1.5 equivalents) was used, from N^2-[4-hydroxy-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (1.0 g, 2.18 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 1.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of acetonitrile and water/1% AcOH (gradient from 20/80 to 50/50), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (240 mg, 24%) as a white solid: ^{1}H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.2 Hz), 0.87 (d, 3H, J= 6.2 Hz), 0.97 (s, 9H), 1.0 (m, 1H), 1.35-1.55 (m, 2H), 2.57 (d, 3H, J= 4.0 Hz), 3.15 (m, 1H), 3.61 (d, 1H, J= 11.4 Hz), 4.31 (d, 1H, J= 9.1 Hz), 7.45 (m, 1H), 7.53 (m, 2H), 7.69 (d, 1H, J= 7.3 Hz), 7.83 (q, 1H, J= 4.0 Hz), 7.87 (d, 1H, J= 8.0 Hz), 7.93 (m, 1H), 8.09 (d, 1H, J= 9.1 Hz), 8.32 (m, 1H), 8.88 (s, 1H), 10.43 (s br, 1H); MS (ESI): 496 (M + Na^+).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (2.0 g, 8.7 mmol) and di-(naphth-1-yl) disulfide[7] (3.0 g), there was obtained 2S-isobutyl-3S-(naphth-1-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3g, 41%): MS (EI): 388 (M^+); the other isomer 2S-isobutyl-3R-(naphth-1-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

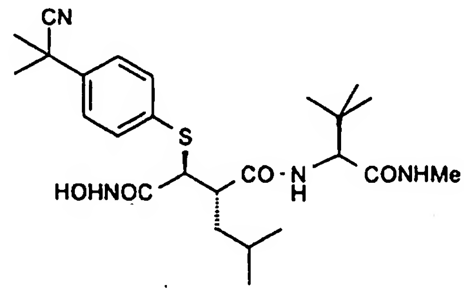
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(naphth-1-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3 g, 3.3 mmol), there was obtained N^2-[2S-isobutyl-3S-(naphth-1-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.26 g, 74%) as a white solid: MS (ESI): 537 (M + Na^+).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(naphth-1-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.2 g, 2.3 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (1.05 g, 100%): MS (ESI): 459 (M + H^+).

[7]Prepared by oxidation of the commercially available thiol in DMSO.

Example 18

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N^2-[4-hydroxy-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)-thiosuccinyl]-L-tert-leucine-N^1-methylamide (1.4 g, 2.9 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 3.0 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 20/80 to 60/40), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)-thiosuccinyl]-L-tert-leucine-N^1-methylamide (140 mg, 10%) as a white solid: m.p. 233-236°C: ^{1}H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.95 (s, 9H), 1.0 (m, 1H), 1.3-1.5 (m, 2H), 1.68 (s, 6H), 2.56 (d, 3H, J= 4.4 Hz), 3.05 (m, 1H), 3.58 (d, 1H, J= 11.4 Hz), 4.24 (d, 1H, J= 9.1 Hz), 7.42 (m, 4H), 7.81 (q, 1H, J=4.4 Hz), 8.03 (d, 1H, J= 9.1 Hz), 9.02 (s br, 1H), 10.68 (s br, 1H); MS (ESI): 513 (M + Na$^+$), 529 (M + K$^+$). The other isomer N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide which was formed in the reaction was not separated from the first isomer and was isolated as a mixture (710 mg, 51%) therewith.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (2.5 g, 10.8 mmol) and di-4-(1-cyano-1-methylethyl)phenyl disulfide[8]

(4.2 g), there was obtained 2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.6g, 38%): ^{1}H-NMR ($CDCl_3$): 0.91 (dd, 6H, J= 6.6 Hz), 1.35 (m, 1H), 1.40 (s, 9H), 1.63 (m, 1H), 1.71 (s, 6H), 1.73 (m, 1H), 2.97 (m, 1H), 3.66 (d, 1H, J= 10.3 Hz), 7.46 (m, 4H); the other isomer 2S-isobutyl-3R-(4-(1-cyano-1-methylethyl)-phenyl)thiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.6 g, 3.9 mmol), there was obtained N^2-[2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thio-4-tert-butyloxy-succinyl]-L-tert-leucine-N^1-methylamide (1.6 g, 77%) as a gum: MS (ESI): 554 ($M + Na^+$).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (1.2 g, 2.3 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)-phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (1.4 g, 100%) as a solid: ^{1}H-NMR (DMSO d-6): 0.79 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.6 Hz), 0.96 (s, 9H), 1.06 (m, 1H), 1.4 (m, 1H), 1.55 (m, 1H), 1.68 (s, 6H), 2.58 (d, 3H, J= 4.4 Hz), 3.06 (m, 1H), 3.64 (d, 1H, J= 11.4 Hz), 3.7 (m br 1H), 4.26 (d, 1H, J= 9.1 Hz), 7.46 (m, 4H), 7.87 (q, 1H, J= 4.4 Hz), 8.13 (d, 1H, J= 9.1 Hz).

[8]Di-4-(1-cyano-1-methylethyl)phenyl disulfide was prepared as follows:

1) alkylation of 4-bromophenylacetonitrile with excess sodium hydride/methyl iodide in THF gave 2-(4-bromophenyl)-2-methylpropionitrile (yield: 90%)

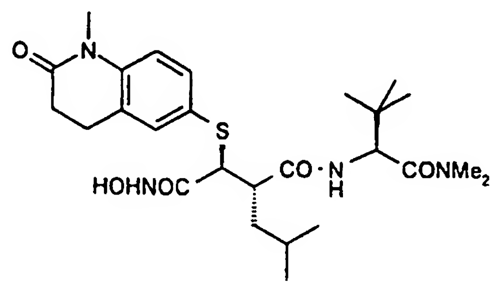
2) 2-(4-tert-butylthiophenyl)-2-methylpropionitrile was obtained from 2-(4-bromophenyl)-2-methylpropionitrile by reaction with tert-butyl mercaptan (1.1 eq.), potassium tert-butoxide (1.35 eq.) in DMSO at 70°C in the presence of $Pd(PPh_3)_4$ (0.03 eq) (yield: 40%)

3) 2-(4-mercaptophenyl)-2-methylpropionitrile was obtained from 2-(4-tert-butylthiophenyl)-2-methylpropionitrile by reaction with trifluoroacetic acid (1.7 eq.) and trifluoromethylsulfonic acid (1 eq.) in thioanisole at room temperature (yield 77%)

4) oxidation of 2-(4-mercaptophenyl)-2-methylpropionitrile in DMSO gave di-4-(1'-cyano-1'-methylethyl)phenyl disulfide (yield: 94%)

Example 19

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-dimethylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N^2- [4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-dimethylamide (450 mg, 0.89 mmol) there was obtained after treatment of the crude reaction mixture with HCl (6N, 0.2 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 20/80 to 60/40), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-dimethylamide (274 mg, 59%) as a white solid: m.p. 240-242°C: ^{1}H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.83 (d, 3H, J= 6.6 Hz), 1.0 (s, 9H), 1.0 (m, 1H), 1.3-1.5 (m, 2H), 2.53 (m, 2H), 2.82 (s, 3H), 2.83 (m, 2H), 3.05 (m, 1H), 3.09 (s, 3H), 3.24 (s, 3H), 3.45 (d, 1H, J= 11.4 Hz), 4.81 (d, 1H, J= 9.2 Hz), 7.03 (d, 1H, J= 8.8 Hz), 7.22 (s, 1H), 7.27 (dd, 1H, J=1.8 Hz, J=8.8 Hz), 8.09 (d, 1H, J= 9.2 Hz), 8.96 (s br, 1H), 10.61 (s br, 1H); MS (ESI): 543 (M + Na^+).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (400 mg, 0.95

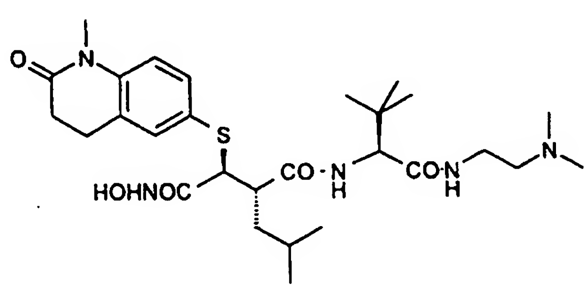
mmol), described in Example 9, and L-tert-leucine dimethylamide[(9)] (165mg, 1.04 mmol) there was obtained N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-dimethylamide (500 mg, 94%) as a white solid: MS (ESI): 584 (M + Na^+).

(ii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-dimethylamide (500 mg, 0.89 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-dimethylamide (450 mg, 100%) as a solid: ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 1.0 (s, 9H), 1.25 (m, 1H), 1.3-1.6 (m, 2H), 2.53 (m, 2H), 2.82 (s, 3H), 2.83 (m, 2H), 3.05 (m, 1H), 3.1 (s, 3H), 3.24 (s, 3H), 3.53 (d, 1H, J= 11.4 Hz), 4.6 (s br, 1H), 4.86 (d, 1H, J= 9.1 Hz), 7.06 (d, 1H, J= 8.8 Hz), 7.26 (s, 1H), 7.33 (m, 1H), 8.19 (d, 1H, J= 9.1 Hz).

[(9)] L-tert-leucine dimethylamide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyl oxazolidine-1,4-dione which was then treated with a saturated solution of dimethylamine in ether.

Example 20

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-(2-dimethylamino)ethylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N^2- [4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-(2-dimethylamino)ethylamide (310 mg, 0.56 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-(2-dimethyl-amino)ethylamide (123 mg, 39%) as a white solid: m.p. 152-158°C; ^{1}H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.99 (s, 9H), 1.1 (m, 1H), 1.3-1.5 (m, 2H), 2.5-2.57 (m, 8H), 2.7-2.75 (m, 2H), 2.83 (m, 2H), 3.04 (m, 1H), 3.24 (s, 3H), 3.33-3.4 (m, 2H), 3.47 (d, 1H, J= 11.4 Hz), 4.24 (d, 1H, J= 8.8 Hz), 7.02 (d, 1H, J= 8.4 Hz), 7.22 (s, 1H), 7.26 (dd, 1H, J= 1.8 Hz, J= 8.4 Hz), 8.1 (m, 2H), 8.98 (s br, 1H), 10.57 (s br, 1H); MS (ESI): 564 ($M + H^+$).

The starting material was prepared as follows:

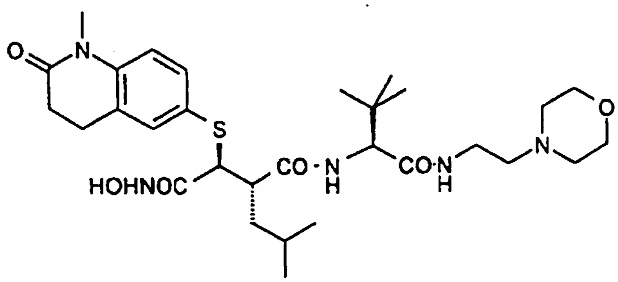
(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (400 mg, 0.95 mmol), described in Example 9, and L-tert-leucine (2-dimethylamino)ethylamide[10] (210mg, 1.04 mmol) there was obtained N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-(2-dimethylamino)ethylamide (369 mg, 64%) as a white solid: MS (ESI): 605 ($M + H^+$).

(ii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-(2-dimethylamino)ethylamide (324 mg, 0.56 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-(2-dimethylamino)ethylamide (430 mg, 100%) as a cream solid: MS (ESI): 548 (M^+).

(10) L-tert-leucine 2-(dimethylamino)ethylamide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with N,N-dimethyl ethylenediamine.

Example 21

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide (237 mg, 0.4 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide (85 mg, 35%) as a white solid: m.p. 156-158°C ;
^{1}H-NMR (DMSO d-6 + TFA) : 0.78 (d, 3H, J = 6.3 Hz), 0.87 (d, 3H, J = 6.3 Hz), 1.0 (s, 9H), 1.0 (m, 1H), 1.41 (m, 2H), 2.51 (m, 2H), 2.84 (m, 2H), 3.08-3.24 (m, 5H), 3.24 (s, 3H), 3.4-3.5 (m, 4H), 3.45 (d, 1H, J = 11.3 Hz), 3.65 (m, 2H), 4.0 (m, 2H), 4.12 (m, 1H), 7.01 (d, 1H, J = 8.5 Hz), 7.21 (d, 1H, J = 2.2 Hz), 7.26 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 8.18 (d, 1H, J = 7.8 Hz); MS (ESI) : 606 (M + H$^+$).

The starting material was prepared as follows:

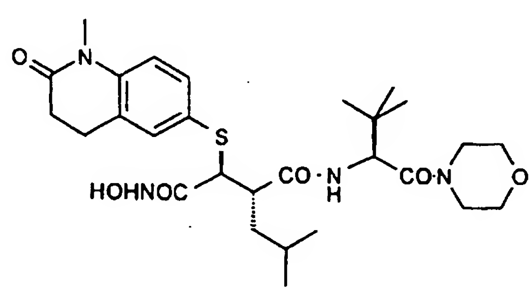
(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (300 mg, 0.71 mmol), described in Example 9, and L-tert-leucine 2-(4-morpholino)ethylamide(11) (190 mg, 0.78 mmol) there was obtained N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide (270 mg, 58%) as a white solid: MS (ESI) : 647 ($M + H^+$) and 669($M + Na^+$).

(ii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide (260 mg, 0.4 mmol) there was obtained N^2-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide (324 mg, 100%) : MS (ESI) : 591 ($M + H^+$).

(11) L-tert-leucine 2-(4-morpholino)ethylamide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with 4-(2-aminoethyl)morpholine.

Example 22

N2-[[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N1-(4-morpholine)amide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N2-[[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-

tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N1-(4-morpholine)amide (415 mg, 0.76 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N1-(4-morpholine)amide (137 mg, 32%) as a white powder : m.p. 218-220°C ; ^{1}H-NMR (DMSO d-6) : 0.76 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 0.94 (m, 1H), 0.99 (s, 9H), 1.35-1.5 (m, 2H), 2.51-2.55 (m, H), 2.8-2.85 (m, 2H), 3.02 (m, 1H), 3.22 (s, 3H), 3.32-3.4 (m, 2H), 3.45 (d, 1H, J = 11.3 Hz), 3.55-3.75 (m, 6H), 4.82 (d, 1H, J = 8.8 Hz), 7.0 (d, 1H, J = 8.4 Hz), 7.2 (d, 1H, J = 1.8 Hz), 7.25 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz), 8.16 (d, 1H, J = 9.1 Hz), 8.95 (s br, 1H), 10.57 (s br, 1H) ; MS (ESI) : 563 (M + H$^+$) and 585 (M + Na$^+$).

The starting material was prepared as follows:

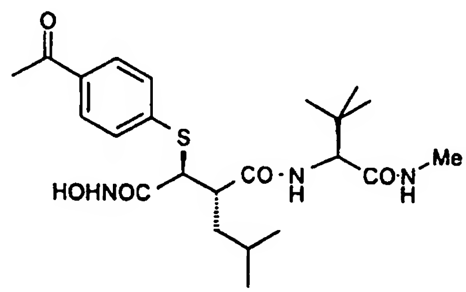
(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-butan-1,4-dioic acid 4-tert-butyl ester (375 mg, 0.89 mmol), described in Example 9, and N-(L-tert-leucine)-(4-morpholine)amide [(12)] (196 mg, 0.98 mmol) there was obtained N-[[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydro-quinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine]-N1-(4- morpholine)amide (465 mg, 86%) as a white solid: MS (ESI) : 626 (M + Na$^+$).

(ii) In a manner analogous to that described in Example 1 (iii), from N-[[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine]-N1-(4-morpholine)amide (460 mg, 0.76 mmol) there was obtained N-[[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N1-(4-morpholine (417 mg, 100%) : MS (ESI) : 548 (M + H$^+$), 570 (M + Na$^+$).

[(12)] N-(L-tert-leucine)-(4-morpholine)amide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with morpholine.

Example 23

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl) hydroxylamine (1.2 equivalents) was used instead of hydroxylamine hydrochloride, from a mixture of N^2- [4-hydroxy-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide and the corresponding 3R epimer (400 mg, 0.89 mmol), there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 30/70 to 60/40), N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (80 mg, 20%) as a white solid : m.p. 210-211°C; ^{1}H-NMR (DMSO d-6) : 0.79 (d, 3H, J = 6.6 Hz), 0.87 (d, 3H, J = 6.6 Hz), 0.9 (s, 9H), 1.04 (m, 1H), 1.39 (m, 1H), 1.49 (m, 1H), 2.56 (s, 3H), 2.57 (d, 3H, J= 3.7 Hz), 3.09 (m, 1H), 3.82 (d, 1H, J = 11.0 Hz), 4.2 (d, 1H, J = 9.2 Hz), 7.46 (d, 2H, J = 8.4 Hz), 7.8 (m, 1H), 7.85 (d, 2H, J = 8.4 Hz), 8.07 (d, 1H, J = 9.2 Hz), 9.07 (s br, 1H), 10.89 (s br, 1H) ; MS (ESI) : 488 (M + Na$^+$),

and the other epimer, N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide (183 mg, 44%) as a white solid : m.p. 220-221°C ; MS (ESI) : 466 (M + H$^+$), 488 (M + Na$^+$).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (5.0 g, 21.7 mmol) and di-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl] disulfide[13] (9.32 g), there was obtained a mixture of 2S-isobutyl-3S-[4-(2-methyl-1,3-

dioxolan-2-yl)phenyl]thiobutan-1,4-dioic acid 4-tert-butyl ester and the corresponding 3R epimer (5.53g, 60%) (3S:3R, 1:3) : MS (ESI): 447 (M + Na$^+$).
This mixture was used unseparated for the next steps.

(ii) In a manner analogous to that described in Example 1 (ii), from the mixture of 2S-isobutyl-3S-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl]thiobutan-1,4-dioic acid 4-tert-butyl ester and the corresponding 3R epimer (2.33 g, 5.49 mmol), there was obtained a mixture of N^2-[2S-isobutyl-3S-{4-(2-methyl-1,3-dioxolan-2-yl)phenyl}thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide together with the corresponding 3R isomer (2.4 g, 79%) as an off white solid: MS (ESI): 551 (M + H$^+$). 573 (M + Na$^+$), 589 (M + K$^+$).

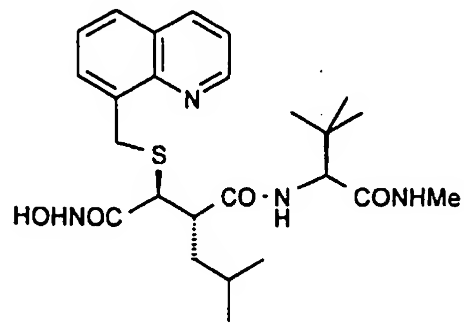
(iii) Hydrochloric acid (2N, 800 μl) was added to a solution of the mixture of N^2-[2S-isobutyl-3S-{4-(2-methyl-1,3-dioxolan-2-yl)phenyl}thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide together with the corresponding 3R isomer (1.74 g, 3.16 mmol) in acetone (17 ml) and the mixture was stirred at room temperature for 4 hours. The acetone was evaporated, the residue taken up in dichloromethane, washed with water and brine and dried over $MgSO_4$. The solvent was removed in vacuo to give a mixture of N^2-[2S-isobutyl-3S-(4-acetylphenyl)-thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide together with the corresponding 3R isomer (1.52 g, 79%) as an off white solid: MS (ESI): 507 (M + H$^+$), 529 (M + Na$^+$).

(iv) In a manner analogous to that described in Example 1 (iii), from mixture of N^2-[2S-isobutyl-3S-(4-acetylphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide together with the corresponding 3R isomer (1.5 g, 2.96 mmol) there was obtained a mixture of N^2-[4-hydroxy-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide and the corresponding 3R epimer (1.17 g, 88%) :MS (ESI): 451 (M + H$^+$), 473 (M + Na$^+$).

(13) Zeneca Ltd., (Bird, T. G. C.; Ple, P.), Patent EP0555068 (1993), in Example 7.

Example 24

N^2-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N^1-methylamide



To a solution of N^2-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N^1-methylamide (460 mg) in DMF (5 ml) cooled at 0°C were added 1-hydroxybenzotriazole (270 mg), N-methylmorpholine (400 μl), O-(2,4-dimethoxybenzyl)hydroxylamine[(12)] (366 mg) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (382 mg). The mixture was stirred at room temperature for 18 hours. The mixture was partitioned between water and ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, brine, dried over $MgSO_4$ and filtered. The solvents were evaporated in vacuo to give crude N^2-[4-(N-(2,4-dimethoxybenzyloxy)amino)-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N^1-methylamide. This crude material was dissolved in dichloromethane (10 ml). Trifluoroacetic acid (0.7 ml) was added. The mixture was stirred at room temperature for 15 minutes. The solvents were evaporated in vacuo. Methanol was added (20 ml) and the solids were filtered off. The filtrate was concentrated and purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 50/50) to give the title compound (76 mg): ^{1}H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (m, 12H), 1.04 (m, 1H), 1.5-1.3 (m, 2H), 2.56 (d, 3H, J= 4.4 Hz), 3.08 (m, 1H), 3.28 (d, 1H, J= 11 Hz), 4.20 (d, 1H, J= 9.2 Hz), 4.43 (d, 1H, J= 12.5 Hz), 4.54 (d, 1H, J= 12.5 Hz), 7.55 (m, 2H), 7.80 (m, 2H), 7.89 (m, 2H), 8.36 (m, 1H), 8.93 (m, 1H), 9.04 (s, 1H), 10.75 (s, 1H); MS (EI): 488 ($M^{+\cdot}$).

[(12)] Prepared from 2,4-dimethoxybenzyl alcohol according to Grochowski E, Jurczak J; Synthesis, 1976, 682.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (400 mg, 1.74 mmol) and di-(quinolin-8-yl)methyl disulfide[13] (726 mg, 2.08 mmol), there was obtained 2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiobutan-1,4-dioic acid-4-tert-butyl ester (487 g, 1:1) contaminated with ca 20% of starting carboxylic acid. MS (ESI): 404 ($M + H^+$).

[13e] Prepared from 8-mercaptomethylquinoline (Chem. Abstracts, 1961, 14458h)

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiobutan-1,4-dioic acid-4-tert-butyl ester (450 mg, 1.12 mmol), there was obtained N^2-[2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (451 mg, 1:1).

(iii) In a manner analogous to that described in Example 1 (iii), from N^2-[2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N^1-methylamide (450 mg) there was obtained N^2-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N^1-methylamide as the trifluoroacetate salt : MS (EI): 474 ($M + H^+$).

Example 25

Typical tablet formulations for a compound of this invention or a pharmaceutically-acceptable salt thereof ('Compound X') are:

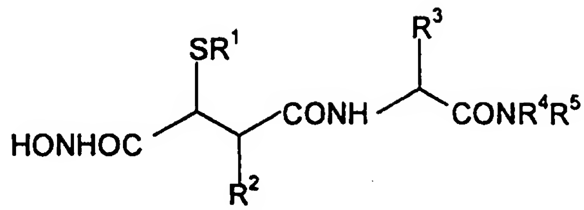
(a)	Tablet Formulation 1	mg/tablet
	Compound X	100
	Lactose Ph.Eur	179
	Croscarmellose sodium	12
	Polyvinylpyrrolidone	6
	Magnesium stearate	3

(b) Tablet Formulation II	mg/tablet
Compound X	250
Lactose Ph.Eur	215
Croscarmellose sodium	20
Polyvinylpyrrolidone	10
Magnesium stearate	5

The tablets may be prepared by conventional procedures well known in the pharmaceutical art and may be film coated with typical coating materials such as hydroxypropylmethylcelluose.

CLAIMS

A compound of the formula (I):



(I)

wherein:

R^1 is aryl, arylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6}alkyl;

R^2 is hydrogen, C_{1-8}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{3-8}cycloalkyl, heteroaryl, heterocyclyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl, heterocyclylC_{1-6}alkyl or C_{3-8}cycloalkylC_{1-6}alkyl;

R^3 is C_{1-6}alkyl, C_{2-6}alkenyl, aryl, C_{1-6}alkyl, heteroarylC_{1-6}alkyl or the side-chain of a naturally occurring amino acid;

R^4 is hydrogen, C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{4-8}cycloalkenyl, arylC_{1-6}alkyl, heteroarylC_{1-6}alkyl or heterocyclylC_{1-6}alkyl;

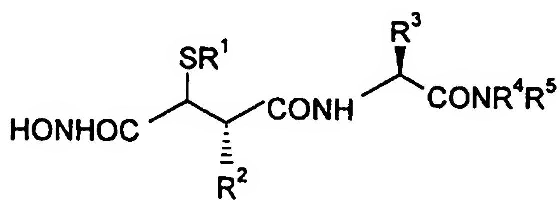
R^5 is hydrogen or C_{1-6}alkyl; or R^4 and R^5 together with the nitrogen atom to which they are joined form a heterocyclic ring;

wherein any group or ring, in R^1-R^5, is optionally substituted;
or a pharmaceutically acceptable salt or an in vivo hydrolysable ester thereof.

2. A compound according to claim 1 wherein R^1 is phenyl, phenylC_{1-6}alkyl, naphthylC_{1-6}alkyl, heteroaryl or heteroarylC_{1-6}alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen, C_{1-6}alkylcarbonyl, C_{1-6}alkylsulfonyl, trifluoromethyl, cyano, C_{1-6}alkyl, C_{1-6}alkoxy, cyanoC_{1-6}alkyl, or two adjacent carbon atoms on a phenyl ring are linked to form a methylenedioxy group.

3. A compound according to claim 1 wherein R^1 is phenyl and two adjacent carbon atoms are linked by $-(CH_2)_m-$ wherein m is 3 or 4, by $-NR^a-CO-(CH_2)_n-$ wherein R is hydrogen or C_{1-6}alkyl and n is 1 or 2, by $-NR^a-COCH=CH-$, $-CO-NR^a-(CH_2)_n-$ or by $-CONR^a-CH=CH-$.

4. A compound according to claim 1 which is of the formula (II):



(II)

wherein R^1 is phenyl unsubstituted or substituted by one or two groups selected from halogen C_{1-6}alkylsulphonyl, trifluoromethyl, C_{1-6}alkyl, C_{1-6}alkoxy, cyano, C_{1-6}alkanoyl, cyanoC_{1-6}alkyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-OCH_2O-$) group; R^2 is isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyldimethylaminoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4 and R^5 together with the nitrogen atom to which they are joined form a morpholino ring.

5. A compound according to claim 1 wherein R^1 is quinolinyl, isoquinolinyl, 1-methyl-2-oxodihydroquinolinyl, 1-methyl-2-oxotetrahydroquinolinyl, 2-methyl-1-oxodihydro-isoquinolinyl or 2-methyl-1-oxotetrahydroisoquinolinyl; R^2 is isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4 and R^5 together with the nitrogen atom to which they are joined form a morpholine ring.

6. A compound according to claim 1 which is:

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-2'-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-dimethylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-(2-dimethylaminoethyl)amide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N^1-[2-(4-morpholino)ethyl]amide;

N^2-[[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N^1-(4-morpholine)amide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N^1-methylamide;

N^2-[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N^1-methylamide;

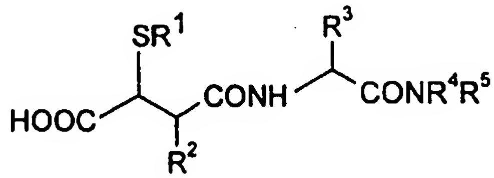
or a pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition which comprises a compound according to any one of claims 1 to 6 and a pharmaceutically acceptable carrier.

8. The use of a compound according to any one of claims 1 to 6 for the manufacture of a medicament for treating disease conditions mediated by TNF.

9. A process for preparing a compound according to any one of claims 1-6 or a pharmaceutically acceptable salt or <u>in vivo</u> hydrolysable ester thereof which process comprises

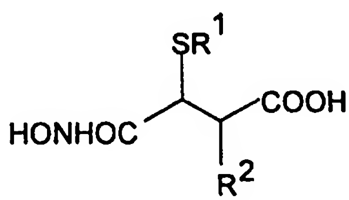
a) reacting a compound of the formula (III):



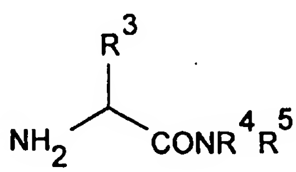
(III)

wherein R^1-R^5 are as defined in claim 1, or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine or a salt thereof; or

b) coupling a compound of the formula (IV) with a compound of the formula (V):



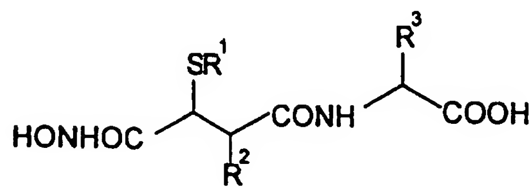
(IV)



(V)

wherein R^1-R^5 are as defined in claim 1, under standard peptide coupling conditions; or

c) reacting a compound of the formula (VI) with compound of the formula (VII):



(VI)

HNR^4R^5 (VII)

wherein R^1-R^5 are as defined in claim 1,

wherein any functional group is protected, if necessary, and:

i. removing any protecting groups;

ii. optionally forming a pharmaceutically acceptable salt or <u>in vivo</u> hydrolysable ester.

10. A compound of the formula (III) as defined in claim 9.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/01164

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C323/22 C07C323/60 C07C323/62 A61K31/16 A61K31/255

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 05719 A (BIRITISH BIOTHECH PHARMACEUTICALS LIM., OXFORD, GB) 31 May 1990	1,2
Y	whole document, especially examples and claims	3-10

X	WO 95 09841 A (BIRITISH BIOTHECH PHARMACEUTICALS LIM., OXFORD, GB) 13 April 1995	1,2
Y	whole document, especially examples and claims	3-10

X	WO 94 10990 A (BIRITISH BIOTHECH PHARMACEUTICALS LIM., OXFORD, GB) 26 May 1994 whole document, especially claim 13	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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2

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 1997	10.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kronester-Frei, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/01164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 613 883 A (YAMANOUCHI PHARMACEUTICAL CO. LTD., TOKYO, JP) 7 September 1994 claims, pages 9/10 ---	1-10
Y	EP 0 236 872 A (F. HOFFMAN-LA ROCHE & CO. AG, BASLE, CH) 16 September 1987 claim 15, page 8, line 29 to page 10, line 14 -----	1-10

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/01164

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9005719 A	31-05-90	AU 644064 B	02-12-93
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Publication Title:

MATRIX METALLOPROTEINASE INHIBITORS

Abstract:

Abstract of WO9944989

The present invention relates to compounds of formula (I) wherein X is a -CO2H or -CONHOH group; Y and Z are independently sulphur or oxygen, at least one being sulphur; R1 is hydrogen, hydroxy, (C1-C6)alkyl, (C2-C6)alkenyl, or (C3-C8)cycloalkyl; R2 is a (C1-C24)alkyl, phenyl(C1-C6)alkyl, or phenyl(C0-C6 alkyl)O(C1-C6)alkyl, any of which may be optionally substituted by (C1-C6)alkyl, (C1-C6)alkoxy, halo or cyano (CN); R3 is the characterising side chain of a natural alpha -amino acid in which any functional groups may be protected, (C1-C6)alkyl which may be optionally substituted, or cycloalkyl(C1-C6)alkyl; R4 is hydrogen, (C1-C6)alkyl, phenyl(C1-C6)alkyl, optionally substituted phenyl or heteroaryl, or a group of formula -(Q-O)n-Q where Q is a straight or branched (C1-C6)alkyl, where n is an integer >1 and no continuous linear sequence of atoms in the gr eea oup R4 is >12; any of the above alkyl or alkenyl being straight or branched; or a salt, hydrate or solvate thereof. The compounds of the invention are useful in the human and veterinary practice. Data supplied from the esp@cenet database - Worldwide

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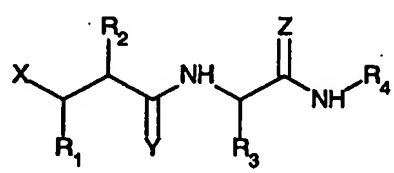

(I)

(57) Abstract

The present invention relates to compounds of formula (I) wherein X is a $-CO_2H$ or $-CONHOH$ group; Y and Z are independently sulphur or oxygen, at least one being sulphur; R_1 is hydrogen, hydroxy, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, or (C_3-C_8)cycloalkyl; R_2 is a (C_1-C_{24})alkyl, phenyl(C_1-C_6)alkyl, or phenyl$(C_0-C_6$ alkyl$)O(C_1-C_6)$alkyl, any of which may be optionally substituted by (C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo or cyano (CN); R_3 is the characterising side chain of a natural α-amino acid in which any functional groups may be protected, (C_1-C_6)alkyl which may be optionally substituted, or cycloalkyl(C_1-C_6)alkyl; R_4 is hydrogen, (C_1-C_6)alkyl, phenyl(C_1-C_6)alkyl, optionally substituted phenyl or heteroaryl, or a group of formula $-(Q-O)_n-Q$ where Q is a straight or branched (C_1-C_6)alkyl, where n is an integer >1 and no continuous linear sequence of atoms in the group R_4 is >12; any of the above alkyl or alkenyl being straight or branched; or a salt, hydrate or solvate thereof. The compounds of the invention are useful in the human and veterinary practice.

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Matrix Metalloproteinase Inhibitors

This invention comprises new matrix metalloproteinase inhibitors, which are succinamide based hydroxamic acid or carboxylic acid thioamides. The invention further comprises processes for their preparation, pharmaceutical compositions containing them, and the use of such compounds in medicine. In particular, the compounds are inhibitors of matrix metalloproteinases involved in tissue degradation. Some of the compounds of the invention are, in addition, inhibitors of the release of tumour necrosis factor- α (TNF-α) from cells.

Background of the Invention

Matrix metalloproteinases (MMPs) are a family of zinc endopeptides, which exhibit proteolytic activity towards most if not all of the constituents of the extracellular matrix, such as the interstitial and basement membrane collagens, fibronectin, and laminin. They play a key role in both physiological and pathological tissue degradation.

At least 16 different and yet highly homologous MMP-species have been characterised. They share a catalytic domain with the HisGluXaaGlyHis motif responsible for ligating zinc, which is essential for the catalytic function. MMP family members differ from each other structurally by the presence or absence of additional domains that contribute to activities, such as substrate specificity, inhibitor binding, matrix binding and cell-surface localisation. [H. Birkedal-Hansen; W. G. Moore, M. K. Bodden: C. J. Windsor; B. Birkedal-Hansen; A. DeCarlo: *Crit. Rev. Oral Biol. Med.* (1993) 4, 197-250 and A. F. Chambers; L. M. Matristan: *J. Natl. Cancer Inst.* (1997) 89(17), 1260-1270]. There are three major groups of MMPs, identified by their substrate preferences: collagenases degrade fibrillar collagen, stromelysins prefer proteoglycans and glycoproteins as substrates and gelatinases are particularly potent in degradation of nonfibrillar and denatured collagens (gelatine).

Compounds which have the property of inhibiting the action of matrix metalloproteinases are thought to be potentially useful for the treatment or prophylaxis of conditions involving tissue breakdown and inflammation, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration, and tumour metastasis, invasion and growth. MMP inhibitors are also of potential value in the treatment of neuro-inflammatory disorders, including those involving myelin degradation, for example multiple sclerosis, as well as in the management of angiogenesis dependent diseases, which include arthritic conditions and solid tumour growth as well as psoriasis, proliferative retinopathies, neovascular glaucoma, ocular tumours, angiofibromas and hemangiomas. However, the relative contributions of individual MMPs in any of the above disease states is not yet fully understood.

TNF-α is a cytokine which is produced as a 28-kDa precursor and released in an active 17-kDa form. This active form can mediate a large number of deleterious effects *in vivo*, including inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration of TNF-α can cause cachexia and anorexia; accumulation of excess TNF-α can be fatal.

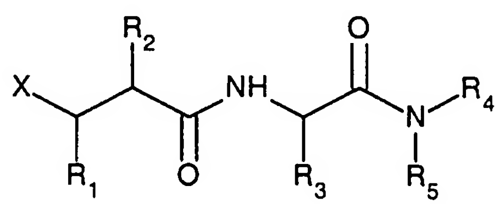
Compounds which inhibit the production or action of TNF-α are therefore thought to be potentially useful for the treatment or prophylaxis of many inflammatory, infectious, immunological and malignant diseases. These include, but are not limited to, septic shock, haemodynamic shock and sepsis syndrome, post ischameic reperfusion injury, malaria, Crohn's disease, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive mono-clonal antibodies and hyperoxic alveolar injury.

TNF-α convertase is a metalloprotease involved in the biosynthesis of TNF-α. Inhibition of TNF-α convertase inhibits production of TNF-α. Since excessive TNF-α production has been noted in several disease conditions characterised by

MMP-mediated tissue degradation, including multiple sclerosis, arthritis and cancer, compounds which inhibit both MMPs and TNF-α production may have particular advantages in the treatment or prophylaxis of diseases or conditions in which both mechanisms are involved.

Many known MMP inhibitors are peptide derivatives, based on naturally occurring amino acids, and are analogues of the cleavage sites in the natural substrates of the MMPs. Other known MMP inhibitors are less peptidic in structure, and may be viewed as pseudopeptides or peptidomimetics. Such compounds usually have a zinc binding group, which most often is a hydroxamic acid, carboxylic acid, sulphhydryl, and oxygenated phosphorous (e.g. phosphinic acid and phosphonamides including aminophosphonic acid) groups.

Two known classes of pseudopeptide or peptidomimetic MMP inhibitors have a hydroxamic acid group and a carboxylic acid group, respectively, as their zinc binding groups. With few exceptions, such known inhibitors may be represented by the structural formula (A)



(A)

in which X is the zinc binding hydroxamic acid (-CONHOH) or carboxylic acid (-COOH) group and the groups R_1 to R_5 are variable in accordance with the specific prior art disclosures of such compounds.

In such compounds, it is generally understood in the art that the variation of the zinc binding group and the substituents R_1, R_2 and R_3 can have an appreciable effect on the relative inhibition of the MMPs. The group X is thought to interact with MMPs by binding to a Zn(II) ion in the active site. Generally the hydroxamic acid is preferred over the carboxylic acid in terms of inhibitory activity against the various MMPs. However, the carboxylic acid moiety in combination with other substituents

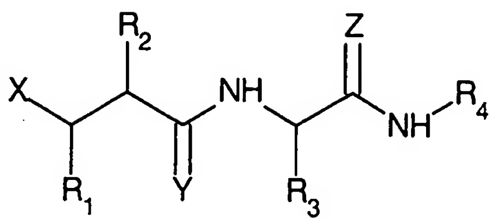
can provide selective inhibition of gelatinase (EP-489,577-A). The R_1, R_2 and R_3 groups are believed to occupy, respectively, the P1, $P1^I$ and $P2^I$ amino acid side chain binding sites for the natural enzyme substrates. There is evidence that a larger R_1 substituent can enhance activity against stromelysin, and that a $(C_1$-$C_6)$alkyl group (such as isobutyl) at R_2 may be preferred for activity against collagenase whilst a phenylalkyl group (such as phenylpropyl) at R_2 may provide selectivity for gelatinase over the other MMPs.

Although numerous MMP inhibitors with potent *in vitro* activities are known, many have not been suitable for further development as medicines, since they have lacked any useful activity when administered orally at pharmaceutically acceptably doses. Although it is known that a number of factors influence oral bioavailability, the design of enzyme inhibitors with high oral bioavailability is far from straightforward. Finding a combination of R_1, R_2, R_3, R_4, or R_5 substituents that permits a good balance of intrinsic level of activity, water solubility, oral absorption, and pharmakokinetic properties is a continuing problem in the art, since those properties can vary in an unpredictable way as the substituents R_1- R_5 are varied. Identifying hydroxamic acid and carboxylic acid based MMP inhibitors having such properties remains a much sought after goal in the art.

Now we have found novel potent hydroxamic acid and carboxylic acid thioamide derivatives that have advantageously good oral bioavailability, and after oral administration have advantageously longer duration of action and a pharmacokinetically better profile than their structurally closely related analogues.

This invention thus relates to a hitherto unknown class of compounds of formula (I) below wherein X is a hydroxamic acid or a carboxylic acid group characterised primarily in that one or both Y and Z groups are the atom S.

The present invention provides compounds of general formula (I)



(I)

wherein

X is a $-CO_2H$ or -CONHOH group;

Y and Z are independently sulphur or oxygen, at least one being sulphur

R_1 is hydrogen, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, or $(C_3\text{-}C_8)$cycloalkyl;

R_2 is a $(C_1\text{-}C_{24})$alkyl, phenyl$(C_1\text{-}C_6)$alkyl, or phenyl$(C_0\text{-}C_6$ alkyl)O$(C_1\text{-}C_6)$alkyl, any of which may be optionally substituted by $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, halo, or cyano (CN);

R_3 is the characterising side chain of a natural α-amino acid in which any functional groups may be protected, $(C_1\text{-}C_6)$alkyl which may be optionally substituted, or cycloalkyl$(C_1\text{-}C_6)$alkyl;

R_4 is hydrogen, $(C_1\text{-}C_6)$alkyl, phenyl$(C_1\text{-}C_6)$alkyl, optionally substituted phenyl or heteroaryl, or a group of formula $-(Q\text{-}O)_n\text{-}Q$ where Q is a straight or branched $(C_1\text{-}C_6)$alkyl, where n is an integer >1 and no continuous linear sequence of atoms in the group R_4 is >12; any of the above alkyl or alkenyl groups being straight or branched;

or a salt, hydrate or solvate thereof.

As used in the specification, unless specified to the contrary, the following terms have the meaning indicated:

"Alkyl" refers to a straight or branched chain alkyl moiety, consisting solely of carbon and hydrogen, containing no unsaturation and having the number of carbon atoms specified, including for example methyl, n-propyl, isobutyl, t-butyl, hexyl and dodecyl.

"(C_2-C_6)alkenyl" refers to a straight or branched chain alkenyl moiety having 2 to 6 carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. This term would include, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

"Alkoxy" refers to a radical of the formula -OR, where R is alkyl as defined above, for example methoxy, n-propoxy, t-butoxy and the like.

"Cycloalkyl" means a saturated alicyclic moiety having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclopentyl, cyclohexyl, and cyclooctyl.

"Characterising side chain of a natural α-amino acid" means the characteristic side chain attached to the $-CH(NH_2)(COOH)$ moiety in the following amino acids: alanine, arginine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine. The amino acid side chains may be protected.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four substituents, each of which independently may be a (C_1-C_6)alkoxy, hydroxy, thio, (C_1-C_6)alkylthio, amino, halo (including fluoro, chloro, bromo and iodo), cyano, trifluoromethyl, nitro, -COOH, $-CONH_2$, $-CONHR^A$ or $-CONR^AR^A$ wherein R^A is a (C_1-C_6)alkyl group or the residue of a natural α-amino acid.

Salts of the compounds of the invention can be formed with bases. Such salts include salts derived from inorganic or organic bases, for example metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium

salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

If the compounds of the invention contain basic moieties, salts may also be formed with pharmaceutically acceptable inorganic or organic acids, such as hydrochloric, hydrobromic, and hydroiodic acid, phosphoric acid, sulphuric acid, nitric acid, p-toluenesulphonic acid, methanesulphonic acid, formic acid, acetic acid, propionic acid, citric acid, tartaric acid, succinic acid, benzoic acid, maleic acid, these examples being considered as non-limiting for the invention.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereoisomers with R or S stereochemistry at each chiral centre. General formula (I), and (unless specified otherwise) all other formulae in this specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures).

In the compounds of the invention, the preferred stereochemistry is in general as follows:

C atom carrying the R_1 and X groups -(S),

C atom carrying the R_2 group -(R),

C atom carrying the R_3 group -(S),

but mixtures in which the above configurations predominate are also contemplated. Without limiting the generality of the foregoing:

Preferred compounds of formula I are those in which X represents -CONHOH.

Examples of particular R_1 groups include hydrogen, hydroxy, methyl, ethyl, n-propyl , allyl and methoxy. Presently preferred are compounds in which R_1 is hydrogen, hydroxyl, allyl or propyl.

Examples of particular R_2 groups include $(C_4\text{-}C_{24})$alkyl, phenyl$(C_1\text{-}C_6)$alkyl. Presently preferred are compounds in which R_2 is isobutyl, phenylpropyl, (4-chlorophenyl)propyl, (4-methylphenoxy)ethyl or $(C_6\text{-}C_{16})$alkyl.

Examples of particular R_3 groups include benzyl, 4-methoxybenzyl, isobutyl, t-butyl, cyclohexylmethyl, indolmethyl, 1-fluoromethylethyl, isopropyl. Presently

preferred are compounds in which R_3 is benzyl, t-butyl, cyclohexylmethyl, 4-methoxybenzyl, indolmethyl, isobutyl or isopropyl.

Examples of particular R_4 groups include $(C_1$-$C_6)$alkyl, phenyl$(C_1$-$C_6)$alkyl and a polyether chain possessing at least two non-adjacent oxygen atoms. Presently preferred are compounds in which R_4 is methyl, phenylpropyl, 2-(2-methoxyethoxy)ethyl, 2-(2-methoxyethoxymethoxy)ethyl or 2-(ethoxyethoxymethoxy)ethyl.

Examples of the invention are:

N^4-Hydroxy-2(R)-phenylethyl-*N*^{1}-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide

N^4-Hydroxy-2(R)-isobutyl-*N*^{1}-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide

N^4-Hydroxy-2(R)-isobutyl-*N*^{1}-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-succinamide

N^4-Hydroxy-*N*^{1}-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-2(R)-phenylpropyl-succinamide

N^4-Hydroxy-2(R)-phenylpropyl-*N*^{1}-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide

N^4-Hydroxy-2(R)-phenylpropyl-*N*^{1}-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamide

N^4-Hydroxy-*N*^{1}-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-2(R)-phenylpropyl-succinamide

N^4-Hydroxy-2(R)-isobutyl-N^1-thiono-N^1-[1(S)-(methylcarbamoyl)-2-phenylethyl]-succinamide

3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-succinamide

3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-succinamide

N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamide

N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-cyclohexylethyl]-succinamide

N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide

N^4-Hydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-phenylethyl}-2(R)-phenylpropyl-succinamide

3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamide

3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2,2-dimethyl-propyl}-succinamide

3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide

N[4]-Hydroxy-2(R)-isobutyl-*N*[1]-[1(S)-(methylthiocarbamoyl)-2-methyl-propyl]-3(S)-propyl-succinamide

N[4]-Hydroxy-2(R)-isobutyl-*N*[1]-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-3(S)-propyl-succinamide

2(R)-Dodecyl-*N*[4]-hydroxy-*N*[1]-[1(S)-(methylthiocarbamoyl)-3-methyl-butyl]-succinamide

2(R)-Dodecyl-*N*[4]-hydroxy-*N*[1]-[1(S)-(phenylethylthiocarbamoyl)-2-methyl-butyl]-succinamide

2(R)-Hexadecyl-*N*[4]-hydroxy-*N*[1]-[1(S)-(phenylthiocarbamoyl)-ethyl]-succinamide

2(R)-Hexadecyl-*N*[4]-hydroxy-*N*[1]-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamide

3(S), *N*[4]-Dihydroxy-*N*[1]-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)-ethyl}-2(R)-phenylpropyl-succinamide

3(S), *N*[4]-Dihydroxy-*N*[1]-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-phenylpropyl-succinamide

N[4]-Hydroxy-2(R)-(4-chlorophenyl)propyl-*N*[1]-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide

N[4]-Hydroxy-2(R)-(4-chlorophenyl)propyl-*N*[1]-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-succinamide

N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-succinamide

N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide

N^4-Hydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-(4-methylphenoxy)ethyl-succinamide

N^4-Hydroxy-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-(4-methoxyphenyl)ethyl)-2(R)-(4-methylphenoxy)ethyl-succinamide

N^4-Hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-2(R)-(4-methylphenoxy)ethyl-succinamide

Examples of prior art patent publications are given below:

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EP-A-0489577(Celltech)
WO 96/16931 (British Biotech)
WO 96/33991 (Sankyo)

The general formula (A) of the prior art patent publications depicts simple peptidic compounds as compared to the thiopeptides of general formula (I) of the present invention. It has now surprisingly been found that not only do the compounds of the present invention have enhanced stability toward enzymatic degradation as compared to that of their oxygenated counterpart, but are also more potent inhibitors than compounds of the prior art publications.

The compounds were tested *in vitro* using the following procedure: matrix metalloproteinases were obtained from culture media conditioned by MCF-7 human breast cancer cells and separated by electrophoresis on SDS-acrylamide gels (7.0%) copolymerised with gelatine (1mg/ml, Sigma, MO, USA). The gels containing the MMPs were then incubated with the test compounds overnight in 10 ml buffer (50 mM tris-HCl, pH 7.5, 200mM NaCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.002% NaN_3) at 37°C. The gels were stained for 60 min with 0.5% Coomassie brilliant blue R-250 in 10% acetic acid, destained with 10% acetic acid, incubated with 5% glycerol, and dried with a gel drier. The molar concentrations that inhibited approximately half of the maximal enzymatic activity were then determined. The results for some of the compounds of the invention and a comparator compound selected from one of the prior art publications listed above (EP-A-0214639) are shown in Table 1.

Table 1. Inhibition of MMPs 2/3 and 9 *in vitro* by compounds of the following examples of the present invention and comparators.

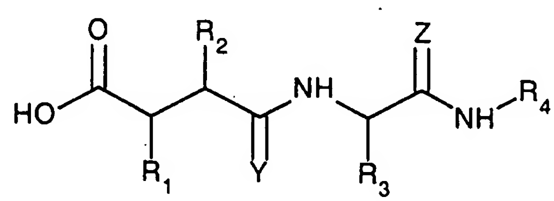
	In vitro inhibition of matrix metalloproteinases (nM)	
Compound	MMP-2/3	MMP-9
Example 3	$1x10^{-9}$	$1x10^{-9}$
Example 4	$1x10^{-9}$	$1x10^{-9}$
Example 5	$1x10^{-9}$	$1x10^{-9}$
Comparator 1	$1x10^{-8}$	$1x10^{-8}$

Comparator 1: N^1-Hydroxy-3R-isobutyl- N^4-[1S-(methylcarbamoyl)-2-phenylethyl]-succinamide (EP-A 0214639).

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. The compounds of the present invention can be synthesised using the methods outlined below, together with methods known in the art of synthetic organic chemistry, or variations thereof as appreciated by those skilled in the art. Preferred methods include, but are not limited to, those described below.

The novel compounds of formula (I) may be prepared using the reactions and techniques described in this section. The reactions are performed in solvents appropriate to the reagents and materials employed and are suitable for the transformations being effected. Also, in the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of experiment and work-up procedures, are chosen to be conditions of standard for that reaction, which should be readily recognised by one skilled in the art. It is understood by one skilled in the art of organic synthesis that the functionality present on various portions of the educt molecule must be compatible with the reagents and reactions proposed. Not all compounds of formula (I) falling into a given class may be compatible with some of the reaction conditions required in some of the methods described. Such restrictions to the substituents which are compatible with the reaction conditions will be readily apparent to one skilled in the art and alternate methods can be used.

Compounds according to the present invention in which X is a hydroxamic acid group -CONHOH may be prepared from compounds of the invention in which X is a carboxylic acid group -COOH. That process, which forms another aspect of the invention, comprises reacting an acid of general formula (II)



(II)

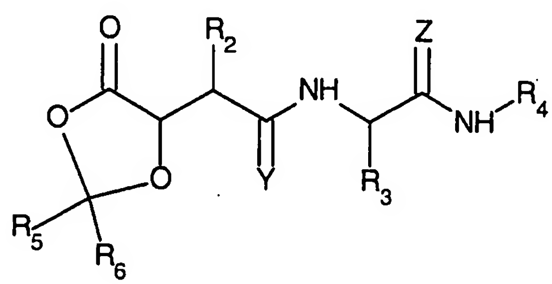
with hydroxylamine, O-protected hydroxylamine, N,O-diprotected hydroxylamine. The acids (II) may themselves be protected from such reaction, then removing any protecting groups from the resulting hydroxamic acid moiety and from any protected substituents in R_1, R_2, R_3, and R_4.

The condensation is carried out using any of the many methods for the formation of amide bonds known to one skilled in the art of organic synthesis. These

methods include, but are not limited to, use of standard coupling procedures such as mixed carbonic anhydride (isobutyl chloroformate) method, carbodiimide (N,N-dimethylaminopropyl-N'-ethyl carbodiimide (EDC), dicyclohexyl carbodiimide, diisopropyl carbodiimide) method, active ester (pentafluorophenyl ester, p-nitrophenyl ester, N-hydroxysuccinic imido ester) method, carbonyldiimidazole method, azide method, phosphorous reagents such as BOP-Cl, azide method, conversion of acid (II) to an acid chloride. Some of these methods (especially carbodiimide) can be enhanced by the addition of 1-hydroxybenzotriazole (HOBt).

Protecting groups as referred to above are well known *per se*, for example from the techniques of peptide chemistry. Amino groups can often be protected by *tert*-butyloxycarbonyl, benzyloxycarbonyl or acetyl groups, or in the form of a phtalimido group. Hydroxy groups are often protected as readily cleavable ethers such as the t-butyl or benzyl ether, or as readily cleavable esters such as the acetate, Carboxylic acid groups are often protected as readily cleavable esters such as the t-butyl or benzyl ester.

In the special case where R_1 in compound (I) is hydroxy, it too may be protected during the coupling of compounds (II). In that case a particularly useful technique may be simultaneous protection of the hydroxy group R_1 and the adjacent carboxyl group as a dioxalone of formula (IIa):

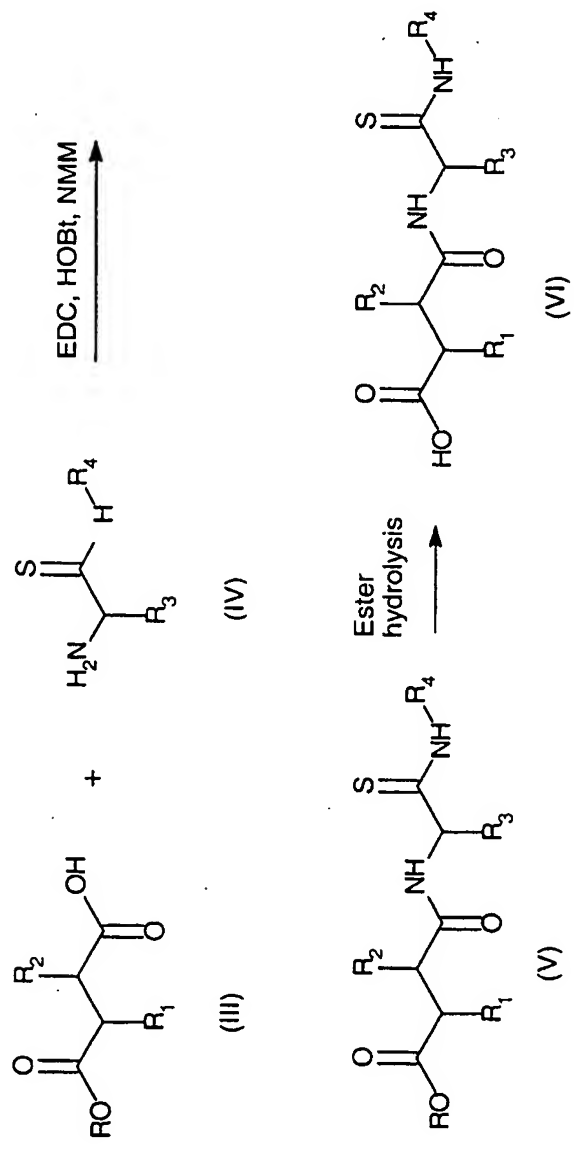


(IIa)

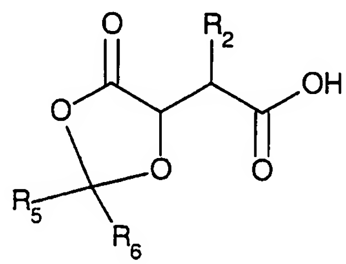
wherein the groups R_5 and R_6 are derived from a dioxalone forming reagent, and may be, for example, hydrogen, alkyl, phenyl or substituted phenyl. The dioxalone ring is opened on reaction with hydroxylamine to give the required hydroxamic acid derivative of formula (I).

Compounds according to the invention in which X is a carboxylic acid group -COOH, Y is oxygen and Z is sulphur may be prepared by a process comprising: coupling of an acid of formula (III) or an activated derivative thereof with an amine of formula (IV), as shown in Scheme 1, where R is an ester protecting group, and R_1, R_2, R_3, and R_4 are as defined in general formula (I), except that any substituents in R_1, R_2,R_3, and R_4 which are potentially reactive in the coupling reaction may themselves be protected from such reaction and the protecting groups subsequently removed. The condensation is carried out using any of the many methods for the formation of amide bonds, as described above.

SCHEME 1



In the special case where R_1 in compound (III) is hydroxy, it too may be protected during the coupling of compounds (III) and (IV). In that case a particularly useful technique may be simultaneous protection of the hydroxy and carboxy groups as a dioxalone of formula (VII), as described above for compounds of general formula (IIa):

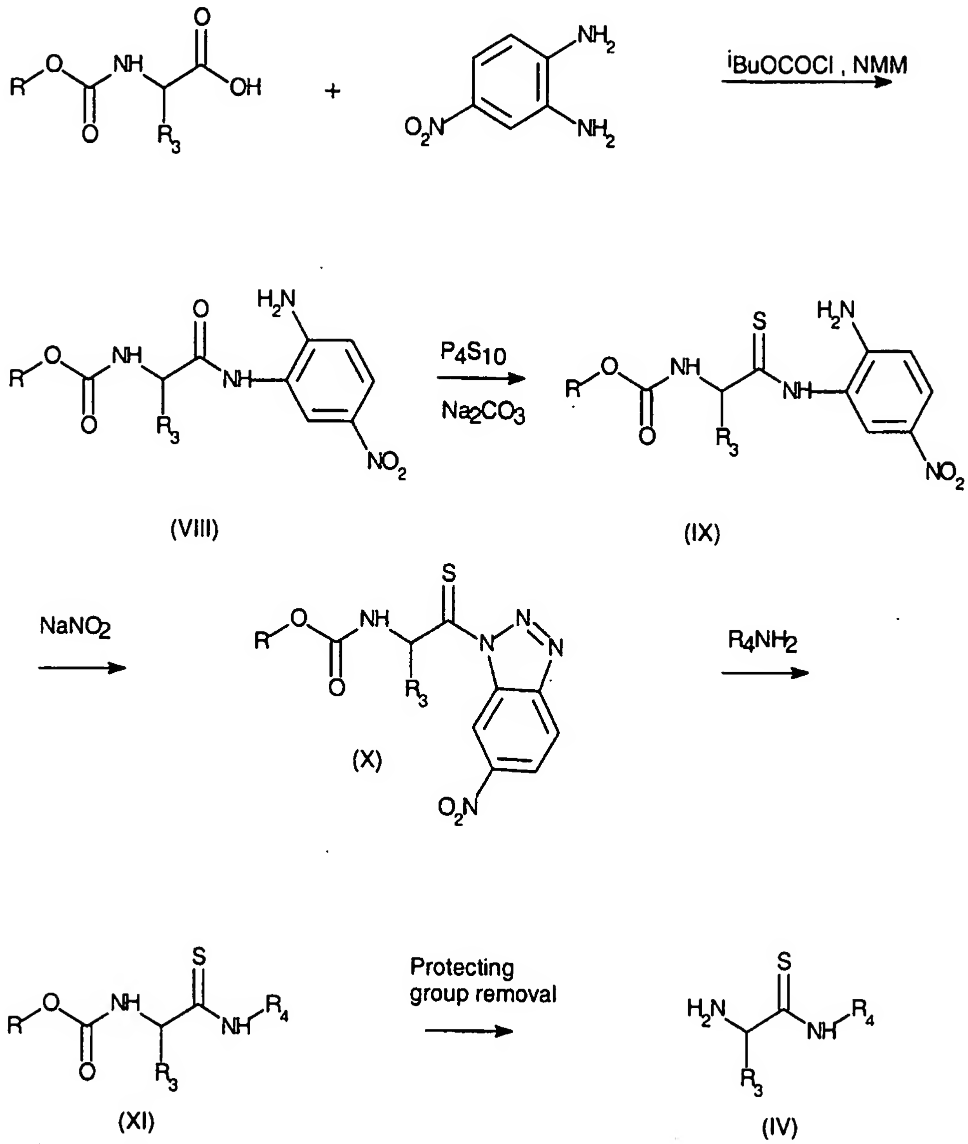


(VII)

The amines of formula (IV) are prepared from the corresponding α-amino acids by methods described in the literature (M. A. Shalaby, C. W. Grote, H. Rapoport; *J. Org. Chem* (1996) **61** 9045-48) and as outlined in Scheme 2 below, in which R is an amine protecting group in the form of a carbamate, for example t-butyl or benzyl.

Starting materials (III) and the α-amino acid starting materials referred to above are either known compounds or prepared by routine known synthetic methods, for example as in the relevant patent publications listed above.

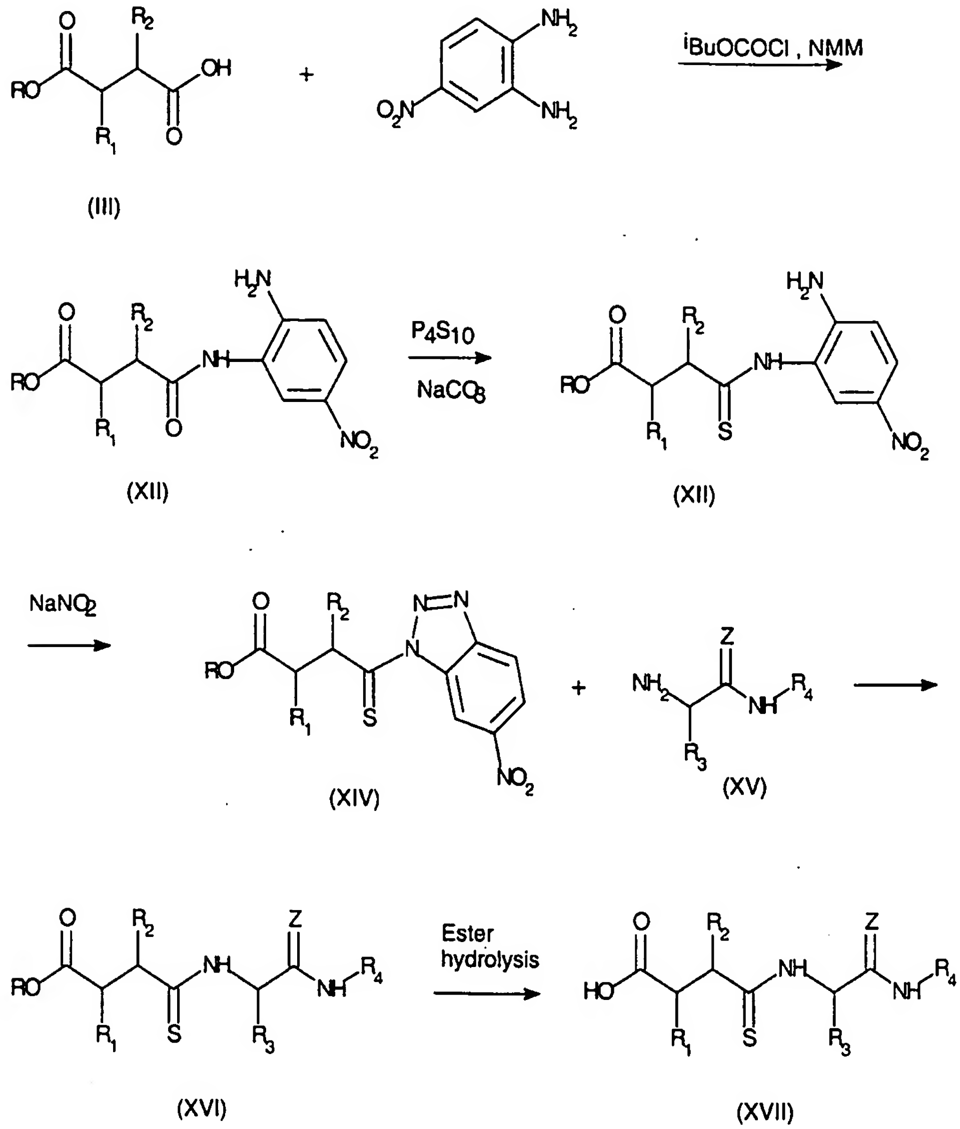
SCHEME2



Compounds according to the invention in which X is a carboxylic acid group -COOH, Y is oxygen and Z is oxygen or sulphur may be prepared by a process comprising: conversion of starting material (III) into an activated species (XIV), which is then allowed to react with amine (XV), as shown in Scheme 3 below.

The present compounds are intended for use in pharmaceutical compositions which are useful in the treatment of the above mentioned diseases.

SCHEME 3



The amount required of a compound of formula I (hereinafter referred to as the active ingredient) for therapeutic effect will, of course, vary both with the particular compound, the route of administration and the mammal under treatment. A suitable dose of a compound of formula I for systemic treatment is 0.1 to 200 mg/kg bodyweight, the most preferred dosage being 0.2 to 50 mg/kg of mammal bodyweight, administered one or more times daily.

While it is possible for an active ingredient, such as a compound according to this invention, to be administered alone as the raw chemical, it is preferable to

administer a compound of the invention as a pharmaceutical formulation. Conveniently, the active ingredient comprises from 0.1% to 100% by weight of the formulation. Conveniently, dosage units of a formulation contain between 0.07 mg and 1 g of the active ingredient, preferably from about 0.5 mg to about 500 mg of the active ingredient, more preferably about 50 mg, e.g. for oral administration. For topical administration, the active ingredient preferably comprises from 1% to 20% by weight of the formulation but the active ingredient may comprise as much as 50% w/w. Formulations suitable for nasal or buccal administration may comprise 0.1% to 20% w/w. for example about 2% w/w of active ingredient.

By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active material as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

The formulations, both for veterinary and human medical use, of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredient(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

The formulations include those in a form suitable for oral, ophthalmic, rectal, parenteral (including subcutaneous, intramuscular and intravenous), transdermal, intra-articular, topical, nasal, or buccal administration.

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing

a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. The active ingredient may also be administered in the form of a bolus, electuary or paste.

Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and a carrier such as cocoa butter, or in the form of an enema.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

Formulations suitable for intra-articular administration may be in the form of a sterile aqueous preparation of the active ingredient which may be in microcrystalline form, for example, in the form of an aqueous microcrystalline suspension. Liposomal formulations or biodegradable polymer systems may also be used to present the active ingredient for both intra articular and ophthalmic administration.

Formulations suitable for topical administration, including eye treatment, include liquid or semi-liquid preparations such as liniments, lotions, gels, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops.

Formulations suitable for administration to the nose or buccal cavity include powder, self-propelling and spray formulations, such as aerosols and atomisers.

In addition to the aforementioned ingredients, the formulations of this invention may include one or more additional ingredients.

The compositions may further contain other therapeutically active compounds usually applied in the treatment.

The invention is further illustrated by the following general procedures, preparations and examples.

<u>General Procedures, Preparations and Examples</u>

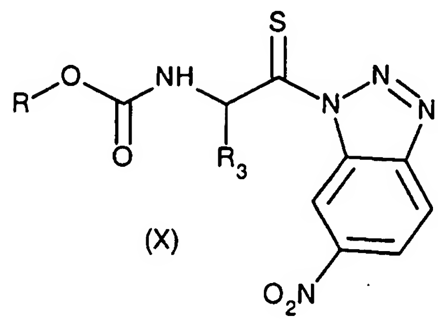
The exemplified compounds are listed in Table 6, compounds of general formula (II) in Table 5, intermediates of general formula (X) in Table 2, intermediates of general formula (IV) in Table 3, and intermediates of general formula (IIa) are listed in Table 4. Compounds of general formula (II) are found in the preparations, not in the examples.

All melting points are uncorrected. For ^{1}H nuclear magnetic resonance (NMR) spectra (300 MHz) and ^{13}C NMR (75.6 MHz) chemical shift values (δ) (in ppm) are quoted, unless otherwise specified, for deuteriochloroform solutions relative to internal tetramethylsilane (δ = 0.00) or chloroform (δ = 7.25) or deuteriochloroform (δ = 76.81 for ^{13}C NMR). The value of a multiplet, either defined (doublet (d), triplet (t), quartet (q)) or not (m) at the approximate mid point is given unless a range is quoted. Mass spectra were recorded on a QUATTRO II (micromass). The organic solvents used were anhydrous. Chromatography was performed on silica gel.

The following abbreviations have been used throughout:

BOC	*tert*-Butyloxycarbonyl
DMF	*N,N*-Dimethylformamide
EDC	*N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt	1-Hydroxybenzotriazole
^{i}Bu	Isobutyl
Me	Methyl
MS	Mass spectroscopy
NMM	*N*-methylmorpholine
NMR	Nuclear magnetic resonance
RT	Room temperature
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

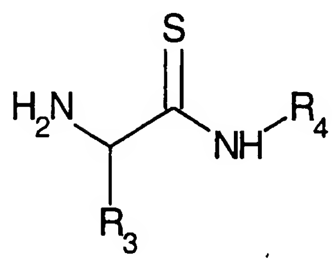
Table 2 Some compounds of general formula (X)



(X)

Compound No.	Preparation No.	R	R_3
201	1	*tert*-butyl	phenylmethyl
202	2	*tert*-butyl	cyclohexylmethyl
203	3	*tert*-butyl	tert-butyl

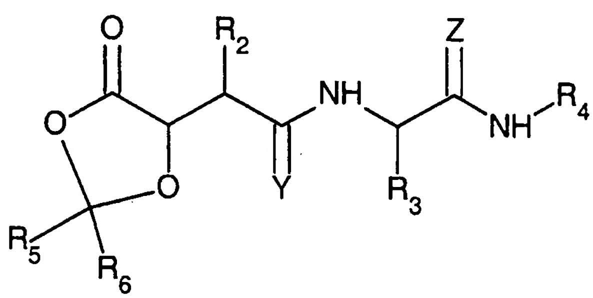
Table 3 Some compounds of general formula (IV)



(IV)

Compound No.	Preparation No.	R_3	R_4
205	5	phenylmethyl	methyl
206	6	phenylmethyl	3-phenylpropyl
207	7	cyclohexylmethyl	methyl
208	8	cyclohexylmethyl	3-phenylpropyl

Table 4 Some compounds of general formula (IIa)



(IIa)

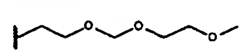
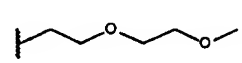
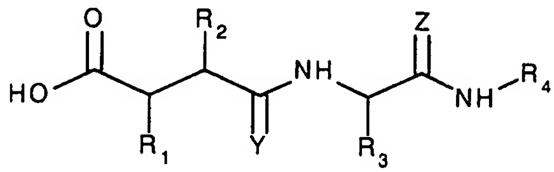
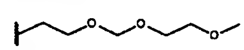
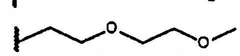
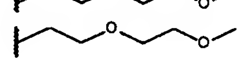
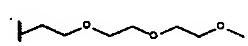
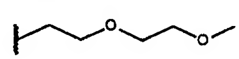
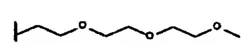
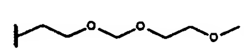
Comp No.	Prep. No.	R_2	R_3	R_4	$R_5 = R_6$	Y	Z
217	17	isobutyl	cyclohexylmethyl	methyl	methyl	O	S
218	18	isobutyl	phenylmethyl	methyl	methyl	O	S
223	23	isobutyl	*tert*-butyl	methyl	methyl	O	S
232	32	phenylpropyl	(4-MeO)-phenylmethyl		methyl	O	S
233	33	phenylpropyl	isopropyl		methyl	O	S

Table 5 Compounds of general formula (II)

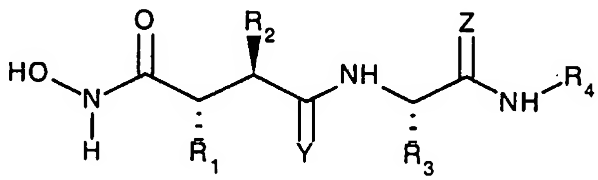


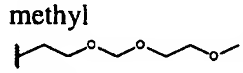
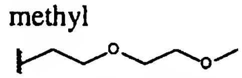
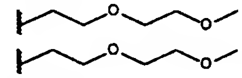
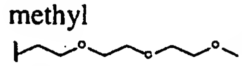
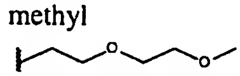
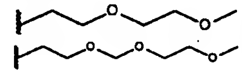
(II)

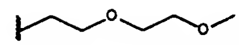
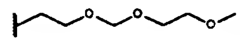
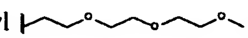
Comp No.	Prep. No.	R_1	R_2	R_3	R_4	Y	Z
209	9	H	2-phenylethyl	phenylmethyl	3-phenylpropyl	O	S
210	10	H	isobutyl	phenylmethyl	3-phenylpropyl	O	S
211	11	H	isobutyl	phenylmethyl	methyl	O	S
212	12	H	3-phenylpropyl	phenylmethyl	methyl	O	S
213	13	H	3-phenylpropyl	phenylmethyl	3-phenylpropyl	O	S
214	14	H	3-phenylpropyl	cyclohexylmethyl	3-phenylpropyl	O	S
215	15	H	3-phenylpropyl	cyclohexylmethyl	methyl	O	S
216	16	H	isobutyl	phenylmethyl	methyl	S	O
219	19	H	Isobutyl	cyclohexylmethyl	3-phenylpropyl	O	S
220	20	H	isobutyl	cyclohexylmethyl	methyl	O	S
221	21	H	isobutyl	indolmethyl	methyl	O	S
222	22	H	3-phenylpropyl	phenylmethyl		O	S
224	24	allyl	isobutyl	*tert*-butyl		O	S
225	25	allyl	isobutyl	4-methoxy-phenylmethyl		O	S
226	26	propyl	isobutyl	isopropyl	methyl	O	S
227	27	propyl	isobutyl	isobutyl		O	S
228	28	H	dodecyl	isobutyl	methyl	O	S
229	29	H	dodecyl	2-butyl	phenylethyl	O	S
230	30	H	hexadecyl	methyl	phenyl	O	S
231	31	H	hexadecyl	*tert*-butyl	methyl	O	S
234	34	H	3-(4-Cl-phenyl)propyl	4-methoxy-phenylmethyl		O	S
235	35	H	3-(4-Cl-phenyl)propyl	isobutyl		O	S
236	36	H	3-(4-Cl-phenyl)propyl	isopropyl		O	S

237	37	H	3-(4-Cl-phenyl) propyl	indolmethyl	methyl	O S
238	38	H	2-(4-Me-phenoxy)ethyl	isopropyl		O S
239	39	H	2-(4-Me-phenoxy)ethyl	4-methoxy-phenylmethyl		O S
240	40	H	2-(4-Me-phenoxy)ethyl	indolmethyl	methyl	O S

Table 6 Exemplified compounds



Comp No.	Ex. No.	R_1	R_2	R_3	R_4	Y	Z
101	1	H	2-phenylethyl	phenylmethyl	3-phenylpropyl	O	S
102	2	H	isobutyl	phenylmethyl	3-phenylpropyl	O	S
103	3	H	isobutyl	phenylmethyl	methyl	O	S
104	4	H	3-phenylpropyl	phenylmethyl	methyl	O	S
105	5	H	3-phenylpropyl	phenylmethyl	3-phenylpropyl	O	S
106	6	H	3-phenylpropyl	cyclohexylmethyl	3-phenylpropyl	O	S
107	7	H	3-phenylpropyl	cyclohexylmethyl	methyl	O	S
108	8	H	isobutyl	phenylmethyl	methyl	S	O
109	9	OH	isobutyl	cyclohexylmethyl	methyl	O	S
110	10	OH	isobutyl	phenylmethyl	methyl	O	S
111	11	H	Isobutyl	cyclohexylmethyl	3-phenylpropyl	O	S
112	12	H	isobutyl	cyclohexylmethyl	methyl	O	S
113	13	H	isobutyl	indolmethyl	methyl	O	S
114	14	H	3-phenylpropyl	phenylmethyl		O	S
115	15	OH	isobutyl	*tert*-butyl	methyl	O	S
116	16	allyl	isobutyl	*tert*-butyl		O	S
117	17	allyl	isobutyl	4-methoxy-		O	S
118	18	propyl	isobutyl	isopropyl	methyl	O	S
119	19	propyl	isobutyl	isobutyl		O	S
120	20	H	dodecyl	isobutyl	methyl	O	S
121	21	H	dodecyl	2-butyl	phenylethyl	O	S
122	22	H	hexadecyl	methyl	phenyl	O	S
123	23	H	hexadecyl	*tert*-butyl	methyl	O	S
124	24	OH	3-phenylproyl	4-methoxy-		O	S
125	25	OH	3-phenylpropyl	isopropyl		O	S

126	26	H	3-(4-Cl-phenyl)	4-methoxy-		O	S
127	27	H	3-(4-Cl-phenyl)	isobutyl		O	S
128	28	H	3-(4-Cl-phenyl)	isopropyl		O	S
129	29	H	3-(4-Cl-phenyl) propyl	indolmethyl	methyl	O	S
130	30	H	2-(4-Me-phenoxy)ethyl	isopropyl		O	S
131	31	H	2-(4-Me-phenoxy)ethyl	4-methoxy-phenylmethyl		O	S
132	32	H	2-(4-Me-phenoxy)ethyl	indolmethyl	methyl	O	S

<u>General Procedure 1: Formation of thioacylating reagents of general formula (X) (cf. Scheme 2).</u>

(M. A. Shalaby, C. W. Grote, H. Rapoport; *J. Org. Chem* (1996) **61** 9045-48). NMM (2.2 ml; 20 mmol) was added to a solution of the N^{α}-BOC amino acid in THF at -20 °C, followed by dropwise addition of isobutyl chloroformiate (1.3 ml, 10 mmol). The mixture was stirred for 30 min, 4-nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the resulting slurry was stirred at -15 °C for 2h and at RT overnight. The mixture was filtered through Celite and the filtrate concentrated. The residue was dissolved in EtOAc, and the solution was washed successively with 1 M $NaHPO_4$, brine, 5% $NaHCO_3$, and brine, then dried ($MgSO_4$) and concentrated. The residue was purified either by crystallisation (EtOAc/ petroleum ether) or chromatography (EtOAc/ petroleum ether) to afford the anilide (VIII).

Under a flow of argon, P_4S_{10} (1.1 g, 2.5 mmol) was mixed with Na_2CO_3(0.27g, 2.5 mmol) in THF (100 ml). The mixture was stirred for 1h at RT and then cooled to 0 °C. To this solution was added anilide (VIII) (5 mmol), and the reaction mixture was stirred at 0°C for 30 min and at RT for 2.5 h. The mixture was filtered through Celite and the filtrate was evaporated. The residue was dissolved in EtOAc and washed twice with 5% $NaHCO_3$, and the aqueous layers were back-extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc/ petroleum ether) to afford the thioanilide (IX). To a solution of thioanilide (IX) (2 mmol) in glacial acetic acid (diluted with 5% water, 15 ml) was added $NaNO_3$ (0.21 g, 3 mmol) in portions over 5 min with stirring. After 30 min, ice water (~100 ml)

was added, and the precipitated product was filtered and washed with water. The solid was dried *in vacuo* overnight and then at 50 °C for 4 h to afford thioacylating reagent (X).

General Procedure 2: Formation of amines of general formula (IV) (cf. Scheme 2).

To a cooled (0 °C) solution of thioacylating reagent (X) (2 mmol) in 30 ml THF was added dropwise a solution of amine R_4NH_2 (2 mmol) in 10 ml THF over a period of 15 min. After 1 h the solvent was evaporated and the residue purified by chromatography (EtOAc/ petroleum ether) to afford the protected amine (XI).

To a solution of protected amine (XI) in CH_2Cl_2 (4 ml) was added dropwise with stirring 4 ml Et_2O saturated with HCl. After 1 h 20 min the precipitated product was filtered off and washed with Et_2O, to yield amine of general formula (IV) as a hydrochloric acid salt.

Alternatively, protected amine (XI) (2 mmol) was dissolved in TFA (20 ml). After 50 min at RT the solution was concentrated, evaporated twice with toluene and once with methanol, to afford amine of general formula (IV) as a trifluoroacetic acid salt.

General Procedure 3: Coupling of acids of general formula (III) with amines of general formula (IV) and subsequent ester hydrolysis (cf. Scheme 1).

To a solution of acid with the general formula (III) (3.4 mmol), amine of general formula (IV) (3.4 mmol, as a hydrochloric or trifluoroacetic acid salt), HOBt (3.4 mmol) and NMM (10.2 mmol) in DMF (20 ml) was added EDC (4.4 mmol) with stirring. The mixture was left at RT overnight and extracted with EtOAC/H_2O. The aqueous layer was back-extracted with EtOAc. The combined organic layers were washed with 2N NaOH, H_2O, 1N HCl, H_2O, brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc/ petroleum ether) to afford the ester of general formula (V). Ester of general formula (V) (2.9 mmol) was subsequently dissolved in formic acid (50 ml) and was left at RT for 1h 20 min,

concentrated, concentrated twice with toluene and once with methanol, to yield acid of general formula (VI).

General Procedure 4: Coupling of acids of general formula (VII) with amines of general formula (IV).

Acid of general formula (VII) (2.4 mmol) was dissolved in CH_2Cl_2 (10 ml) and cooled to 0 °C before adding pentafluorophenol (670 mg, 3.6 mmol) and EDC. (560 mg, 2.9 mmol). The reaction mixture was stirred at 0 °C for 2h then the solution was washed with 1N Na_2CO_3 and brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (CH_2Cl_2) to give a pentafluorophenyl ester.

The pentafluorophenyl ester (2.0 mmol) was dissolved in DMF (2 ml) and cooled to 0 °C before adding amine of general formula (IV) (1.95 mmol). The solution was stirred at 0 °C for 10 min, then overnight at RT. The solvent was evaporated and the residue was dissolved in Et_2O and washed successively with H_2O, 1N Na_2CO_3, H_2O, brine, dried ($MgSO_4$) and concentrated. The residue was purified either by crystallisation or chromatography to afford a compound of general formula (IIa).

General Procedure 5: Formation of thioacylating reagents of general formula (XIV) (cf. Scheme 3).

Thioacylating reagents of general formula (XIV) were formed analogously to thioacylating reagent (X), see General Procedure 1, starting from carboxylic acids of general formula (III).

General Procedure 6: Formation of carboxylic acids of general formula (XVII) (cf. Scheme 3).

To a cooled (0 °C) solution of thioacylating reagent (XIV) (2 mmol) in 30 ml THF was added dropwise a solution of amine (XV) (2 mmol) in 10 ml THF over a period of 15 min. After 1 h the solvent was evaporated and the residue purified by

chromatography (EtOAc/ petroleum ether) to afford the ester of general formula (XVI).

Ester of general formula (XVI) (1.6 mmol) was dissolved in TFA and left at RT for 15 min. The solution was then concentrated, concentrated twice with toluene, once with methanol and purified by chromatography (1-5% methanol in CH_2Cl_2) to yield carboxylic acid of general formula (XVII).

General Procedure 7: Formation of hydroxamic acids of general formula (I) from the corresponding carboxylic acids of general formula (II) or (XVII).

A solution of carboxylic acid with general formula (II) (2.9 mmol) in THF (45 ml) was cooled to -10 °C under argon. NMM (0.3 ml, 3.0 mmol) and isobutyl chloroformate (0.4 ml, 3.0 mmol) were then added with stirring. After 30 min at -10 °C, *O*-trimethylsilyl hydroxylamine (0.4 ml, 3.2 mmol) was added, and the mixture was left at -10 °C for 2h. The mixture was then acidified with 1N acetic acid, extracted with EtOAC/H_2O. The aqueous layer was back-extracted with EtOAc, and the combined organic layers were washed with H_2O, brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography (chloroform: methanol: NH_3 (25%) 95:5:1) or crystallisation to afford the hydroxamic acid of general formula (I).

General Procedure 8: Formation of hydroxamic acids of general formula (I) from the corresponding compounds of general formula (IIa):

To a solution of compound of general formula (IIa) (0.1 mmol) in dichloromethane (2 ml) was added *O*-trimethylsilyl hydroxylamine (0.037 ml, 0.3 mmol). The solution was left overnight and concentrated. The residue was purified by chromatography (chloroform: methanol: NH_3 (25%) 95:5:1) or crystallisation to afford the hydroxamic acid of general formula (I).

General Procedure 9: Formation of hydroxamic acids of general formula (I) from the corresponding carboxylic acids of general formula (II) or (XVII).

To a solution of carboxylic acid of general formula (II) (0.29 mmol) in dry DMF (4.5 ml) was added HOBt (0.38 mmol), NMM (0.38 mmol) and EDC (0.38 mmol). The reaction mixture was cooled to 0 °C and hydroxylamine hydrochloride (0.58 mmol) and NMM (0.58 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred overnight. After addition of ethyl acetate and water, the aqueous phase was separated and washed 3 times with ethyl acetate. The combined organic layers were washed with water and brine, dried and concentrated under reduced pressure. Flash chromatography (chloroform: methanol: NH_3 (25%) 95:5:1) afforded the hydroxamic acid of general formula (I).

Preparation 1: 1-(N^{α}-BOC-L-thionophenylalanine)-6-nitrobenzotriazole (compound 201).

General procedure 1.

Starting material: N^{α}-BOC-L-phenylalanine.

^{13}C NMR ($CDCl_3$) δ 209.0, 155.0, 149.5, 149.0, 135.3, 131.7, 129.4, 128.5, 127.3, 122.2, 121.5, 112.7, 80.5, 62.0, 42.8, 28.3.

Preparation 2: 1-(N^{α}-BOC-L-thionocyclohexylalanine)-6-nitrobenzotriazole (compound 202).

General procedure 1.

Starting material: N^{α}-BOC-L-cyclohexylalanine.

^{13}C NMR ($CDCl_3$) δ 211.9, 155.5, 149.6, 149.0, 132.0, 122.1, 121.4, 112.9, 80.5, 59.7, 44.6, 35.0, 34.1, 32.0, 28.4, 26.4, 26.2, 25.9.

Preparation 3: 1-(N^{α}-BOC-L-thiono-*tert*-leucine)-6-nitrobenzotriazole (compound 203).

General procedure 1.

Starting material: N^{α}-BOC-L-*tert*-leucine.

^{13}C NMR ($CDCl_3$) δ 210.4, 155.5, 149.6, 149.4, 131.4, 122.2, 121.6, 112.9, 80.4, 66.3, 37.1, 28.4, 26.6.

Preparation 4: 1-[2(R)-isobutyl-1-thionosuccinic acid 4-*tert*-butyl ester]-6-nitrobenzotriazole (compound 204).

General procedure 5.

Starting material: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester.

^{13}C NMR ($CDCl_3$) δ 215.6, 170.7, 149.4, 149.4, 131.9, 121.9, 121.3, 113.3, 81.1, 47.6, 46.6, 41.3, 28.0, 26.1, 23.0, 22.4.

Preparation 5: L-thionophenylalanine *N*-methylamide hydrochloric acid salt (compound 205).

General procedure 2.

Starting materials: compound 201 and methylamine.

^{1}H NMR (DMSO-d_6) δ 10.68(bs,1H), 8.46(bs,3H), 7.36-7.18(m,5H), 4.32(m,1H), 3.10(m,2H), 2.84(s,3H).

Preparation 6: L-thionophenylalanine *N*-(3-phenylpropyl)amide trifluoroacetic acid salt (compound 206).

General procedure 2.

Starting materials: compound 201 and 3-phenylpropylamine.

^{13}C NMR (DMSO-d_6) δ 196.8, 141.2, 134.7, 129.4, 128.4, 128.3, 128.2, 127.2, 125.9, 58.7, 44.6, 32.2, 28.2.

Preparation 7: L-thionocyclohexylalanine *N*-methylamide hydrochloric acid salt (compound 207).

General procedure 2.

Starting materials: compound 202 and 3-phenylpropylamine.

^{13}C NMR (DMSO-d_6) δ 198.9, 55.2, 41.3, 32.7, 32.6, 32.2, 31.9, 25.7, 25.4, 25.3.

Preparation 8: L-thionocyclohexylalanine *N*-(3-phenylpropyl)amide hydrochloride salt (compound 208).

General procedure 2.

Starting material: compound 202.

^{13}C NMR (DMSO-d_6) δ 198.3, 141.2, 128.2, 125.8, 55.3, 44.7, 41.3, 32.9, 32.8, 32.4, 32.0, 28.6, 25.7, 25.4, 25.4.

Preparation 9: 2(R)-phenylethyl-*N*-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamic acid (compound 209).

General procedure 3.

Starting materials: 2(R)-phenylethyl-succinic acid 4-*tert*-butyl ester and compound 206.

^{13}C NMR ($CDCl_3$) δ 201.7, 175.9, 174.2, 140.8, 140.8, 136.4, 129.3, 128.6, 128.5, 128.5, 128.4, 128.3, 127.1, 126.2, 126.1, 60.7, 45.3, 41.9, 41.8, 36.4, 33.8, 33.0, 33.0, 28.9.

Preparation 10: 2(R)-isobutyl-*N*-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamic acid (compound 210).

General procedure 3.

Starting materials: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester and compound 206.

^{13}C NMR ($CDCl_3$) δ 201.7, 175.6, 174.8, 140.9, 136.6, 129.3, 128.6, 128.5, 128.3, 127.1, 126.1, 60.7, 45.3, 41.8, 41.2, 40.7, 36.7, 33.0, 28.9, 25.6, 22.6, 22.3.

Preparation 11: 2(R)-isobutyl-*N*-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-succinamic acid (compound 211).

General procedure 3.

Starting materials: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester and compound 205.

^{13}C NMR ($CDCl_3$) δ 202.7, 176.2, 174.8, 136.5, 129.2, 128.5, 127.0, 60.4, 41.9, 41.3, 40.7, 36.7, 32.4, 25.6, 22.6, 22.3.

Preparation 12: *N*-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-2(R)-phenylpropyl-succinamic acid (compound 212).

General procedure 3.

Starting materials: 2(R)-phenylpropyl-succinic acid 4-*tert*-butyl ester and compound 205.

^{1}H NMR ($CDCl_3$) δ 7.75(bq,1H), 7.34-7.07(m,10H), 6.87(d,1H), 4.83(m,1H), 3.13(dd,1H), 3.01(dd,1H), 2.89(d,3H), 2.77-2.31(m,5H), 1.74-1.36(m,4H).

Preparation 13: 2(R)-phenylpropyl-*N*-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamic acid (compound 213).

General procedure 3.

Starting materials: 2(R)-phenylpropyl-succinic acid 4-*tert*-butyl ester and compound 206.

^{1}H NMR ($CDCl_3$) δ 7.38(bt,1H), 7.32-7.01(m,15H), 6.88(d,1H), 4.73(m,1H), 3.43(m,2H), 3.15(dd,1H), 2.98(dd,1H), 2.72-2.50(m,5H), 2.43(t,2H), 1.75-1.40(m,6H).

Preparation 14: 2(R)-phenylpropyl-*N*-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamic acid (compound 214).

General procedure 3.

Starting materials: 2(R)-phenylpropyl-succinic acid 4-*tert*-butyl ester and compound 208.

^{1}H NMR ($CDCl_3$) δ 8.42(t,1H), 7.36-7.06(m,10H), 6.46(d,1H), 4.66(m,1H), 3.73-3.44(m,2H), 2.79-2.49(m,6H), 2.42(dd,1H), 1.91(m,2H), 1.77-0.73(m,17H).

Preparation 15: *N*-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-2(R)-phenylpropyl-succinamic acid (compound 215).

General procedure 3.

Starting materials: 2(R)-phenylpropyl-succinic acid 4-*tert*-butyl ester and compound 207.

^{1}H NMR ($CDCl_3$) δ 8.84(q,1H), 7.32-7.09(m,5H), 6.48(d,1H), 4.77(m,1H), 2.99(d,3H), 2.73(dd,1H), 2.60(m,3H), 2.43(dd,1H), 1.77-0.75(m,17H).

Preparation 16: (R)-isobutyl-*N*-thiono- *N*-[1(S)-(methylcarbamoyl)-2-phenylethyl]-succinamic acid (compound 216).

General procedure 6.

Starting materials: compound 204 and L-phenylalanine *N*-methylamide.

^{13}C NMR ($CDCl_3$) δ 208.6, 177.1, 171.0, 136.5, 129.2, 128.7, 127.1, 60.5, 49.2, 44.1, 40.5, 37.2, 26.2, 25.5, 23.0, 22.1.

Preparation 17: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid [1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-amide (compound 217).

General procedure 4.

Starting materials: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid and compound 207.

^{13}C NMR ($CDCl_3$) δ 205.1, 172.0, 171.1, 111.0, 74.7, 56.6, 47.2, 42.9, 36.8, 34.1, 33.7, 32.6, 32.5, 26.9, 26.4, 26.4, 26.1, 25.8, 25.7, 23.4, 21.7.

Preparation 18: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid [1(S)-(methylthiocarbamoyl)-2-phenylethyl]-amide (compound 218).

General procedure 4.

Starting materials: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid and compound 205.

^{13}C NMR ($CDCl_3$) δ 202.6, 172.2, 170.5, 136.6, 129.1, 128.7, 127.1, 111.2, 74.8, 60.7, 46.8, 41.5, 36.2, 32.5, 26.8, 25.8, 25.6, 23.3, 21.7.

Preparation 19: 2(R)-isobutyl-*N*^{1}-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamic acid (compound 219).

General procedure 3.

Starting materials: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester and compound 208.

^{13}C NMR ($CDCl_3$) δ 204.1, 176.1, 175.1, 141.0, 128.5, 128.4, 126.2, 57.0, 45.4, 42.8, 41.4, 40.6, 36.6, 34.1, 33.4, 33.3, 32.9, 29.3, 26.4, 26.2, 26.0, 25.6, 22.7, 22.3, 15.2.

Preparation 20: 2(R)-isobutyl-*N^{1}*-[1(S)-(3-methylthiocarbamoyl)-2-cyclohexylethyl]-succinamic acid (compound 220).

General procedure 3.

Starting materials: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester and compound 207.

^{1}H NMR ($CDCl_3$) δ 8.70(d,1H), 6.60 (d,1H), 4.78 (q,1H, 3.08 (d,3H),3.10(m,1H), 2.7 (m,2H), 2.5 (m,1H), 1.7 (m,6H),1.2(m,10H), 0.93 (d,3H), 0.88 (d,3H).

Preparation 21: 2(R)-isobutyl-*N^{1}*-[1(S)-(3-methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamic acid (compound 221).

General procedure 3.

Starting materials: 2(R)-isobutyl-succinic acid 4-*tert*-butyl ester and L-thionotryptophane *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.2, 177.9, 176.4, 136.0, 127.4, 123.5, 122.1, 119.5, 118.6, 111.5, 110.3, 60.0, 41.7, 32.6, 31.5, 25.5, 22.5.

Preparation 22: *N^{1}*-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthio-carbamoyl]-2-phenylethyl}-2(R)-phenylpropyl-succinamic acid (compound 222).

General procedure 3.

Starting materials: 2(R)-phenylpropyl-succinic acid 4-*tert*-butyl ester and L-thionophenylalanine *N*-[2-(2-methoxy-ethoxymethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 202.0, 141.7, 136.6, 129.3, 128.5, 128.4, 128.3, 126.9, 125.9, 95.5, 71.8, 66.8, 65.5, 60.5, 58.9, 45.8, 42.5, 42.1, 35.6, 31.7, 29.7, 28.8.

Preparation 23: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid [1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-amide (compound 223).

General procedure 4.

Starting materials: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-4-methylpentanoic acid and L-thiono*tert*butylglycine *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 202.0, 171.9, 170.5, 110.9, 74.8, 65.2, 47.7, 37.0, 35.7, 32.5, 26.9, 26.8, 25.8, 25.7, 23.2, 21.9.

Preparation 24: 3(S)-Allyl-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2,2-dimethyl-propyl}-succinamic acid (compound 224).

General procedure 3.

Starting materials: 3(R,S)-allyl-2(R)-isobutyl-succinic acid-4-*tert*-butyl ester and L-thiono*tert*butylglycine *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 200.8, 175.6, 174.9, 135.1, 117.7, 71.9, 70.3, 68.1, 65.2, 59.0, 47.6, 46.0, 45.5, 39.2, 36.0, 34.6, 27.0, 25.9, 23.7, 21.7.

Preparation 25: 3(S)-Allyl-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamic acid (compound 225).

General procedure 3.

Starting materials: 3(R,S)-allyl-2(R)-isobutyl-succinic acid-4-*tert*-butyl ester and L-thiono-4-methoxy-phenylalanine *N*-[2-(2-methoxy-ethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 201.7, 175.5, 174.4, 158.8, 135.1, 130.3, 128.3, 117.7, 114.0, 71.8, 70.1, 68.1, 60.4, 58.9, 55.2, 47.2, 45.6, 41.4, 38.5, 34.4, 25.9, 23.7, 21.5.

<u>Preparation 26: 2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-methyl-propyl]-3(S)-propyl-succinamic acid (compound 226).</u>

General procedure 3.

Starting materials: 2(R)-isobutyl-3(S)-propyl-succinic acid-4-*tert*-butyl ester and L-thionovaline *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{1}H NMR ($CDCl_3$) δ 9.07 (q,1H), 7.12 (d,1H), 4.53 (t,1H), 3.14 (d,3H),2.60(d,2H), 2.19 (m,1H), 1.65 (m,2H), 1.41 (m,4H),1.05-0.80 (m,17H).

<u>Preparation 27: 2(R)-isobutyl-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-3(S)-propyl-succinamic acid (compound 227).</u>

General procedure 3.

Starting materials: 2(R)-isobutyl-3(S)-propyl-succinic acid-4-*tert*-butyl ester and L-thionoleucine *N*-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 204.3, 177.7, 174.2, 71.9, 70.6, 70.4, 70.3, 68.0, 59.0, 57.1, 48.3, 47.4, 45.6, 45.2, 40.0, 32.9, 25.9, 24.9, 23.7, 22.9, 22.2, 21.6, 20.7, 13.9.

<u>Preparation 28: 2(R)-Dodecyl-N^1-[1(S)-(methylthiocarbamoyl)-3-methyl-butyl]-succinamic acid (compound 228).</u>

General procedure 3.

Starting materials: 2(R)-dodecyl-succinic acid-4-*tert*-butyl ester and L-thionoleucine *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 205.0, 177.2, 175.4, 57.5, 44.4, 42.9, 36.9, 32.7, 32.5, 31.9, 29.7, 29.6, 29.5, 29.4, 27.2, 24.8, 22.8, 22.7, 22.1, 14.1.

Preparation 29:2(R)-Dodecyl-*N*[1]-[1(S)-(phenylethylthiocarbamoyl)-2-methyl-butyl]-succinamic acid (compound 229).

General procedure 3.

Starting materials: 2(R)-dodecyl-succinic acid-4-*tert*-butyl ester and L-thionoisoleucine *N*-phenylethylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.5, 176.8, 175.3, 138.2, 128.6, 126.6, 63.6, 46.7, 43.0, 39.2, 37.1, 33.6, 32.7, 31.9, 29.7, 29.6, 29.4, 27.3, 24.8, 22.7, 15.3, 14.1, 10.9.

Preparation 30: 2(R)-Hexadecyl-*N*[1]-[1(S)-(phenylthiocarbamoyl)-ethyl]-succinamic acid (compound 230).

General procedure 3.

Starting materials: 2(R)-hexadecyl-succinic acid-4-*tert*-butyl ester and L-thionoalanine *N*-phenylamide hydrochloric acid salt (prepared as described for compound 205).

^{1}H NMR ($CDCl_3$) δ 10.67(s,1H), 7.76 (d,2H), 7.36 (t,2H), 7.22 (t,1H),6.96(d,1H), 5.16 (m,1H), 2.68 (m,2H), 2.45 (m,1H),1.62(m,1H), 1.49 (d,3H), 1.25 (m,30H), 0.87 (t,3H).

Preparation 31: 2(R)-Hexadecyl-*N*[1]-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamic acid (compound 231).

General procedure 3.

Starting materials: 2(R)-hexadecyl-succinic acid-4-*tert*-butyl ester and L-thiono*tert*butylglycine *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{1}H NMR (DMSO-d_6) δ 12.7(s,1H), 10.08 (q,1H), 7.37 (d,1H), 4.62 (d,1H),2.94(d.3H), 2.74 (m,1H), 2.4 (dd,1H), 2.25 (dd,1H),1.40(m,1H), 1.35-1.05 (m,29H), 0.92 (s,9H), 0.85 (t,3H).

Preparation 32: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-(3-phenyl)propanoic acid {1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)-ethyl}-amide (compound 232).

General procedure 4.

Starting materials: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-5-phenylpentanoic acid and L-thiono-4-methoxy-phenylalanine *N*-[2-(2-methoxy-ethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 201.9, 172.0, 169.8, 158.7, 141.8, 130.3, 128.6, 128.4, 128.4, 125.9, 114.0, 111.0, 74.4, 71.8, 70.2, 68.0, 61.2, 59.0, 55.2, 49.0, 45.4, 41.2, 35.6, 28.8, 27.4, 26.9, 25.9.

Preparation 33: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-(3-phenyl)propanoic acid {1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-amide (compound 233).

General procedure 4.

Starting materials: 2(R)-(2,2-dimethyl-5-oxo-[1,3]dioxalan-4(S)-yl)-5-phenylpentanoic acid and L-thiono-valine *N*-[2-(2-methoxy-ethoxymethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.6, 172.1, 170.2, 141.8, 128.4, 128.3, 125.8, 110.8, 95.9, 74.4, 71.9, 67.0, 66.2, 64.3, 59.1, 49.5, 46.0, 35.6, 33.9, 28.7, 27.5, 27.0, 26.0, 19.5, 18.3.

Preparation 34: 2(R)-(4-chlorophenyl)propyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamic acid (compound 234).

General procedure 3.

Starting materials: 2(R)-(4-chlorophenyl)propyl-succinic acid-4-*tert*-butyl ester and L-thiono-4-methoxy-phenylalanine *N*-[2-(2-methoxy-ethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 202.0, 174.7, 174.1, 158.7, 140.1, 131.6, 130.3, 129.8, 128.6, 128.5, 114.0, 71.8, 70.1, 68.1, 61.0, 58.9, 55.2, 45.6, 42.6, 41.1, 36.4, 34.9, 31.6, 28.6.

<u>Preparation 35: 2(R)-(4-chlorophenyl)propyl-*N*^{1}-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-succinamic acid (compound 235).</u>

General procedure 3.

Starting materials: 2(R)-(4-chlorophenyl)propyl-succinic acid-4-*tert*-butyl ester and L-thionoleucine *N*-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 204.5, 175.1, 174.5, 140.2, 131.6, 129.8, 128.4, 71.9, 70.5, 70.3, 70.2, 68.1, 58.9, 57.5, 45.6, 44.9, 42.5, 36.5, 34.9, 31.8, 28.7, .4.8, 22.9, 22.1.

<u>Preparation 36: 2(R)-(4-chlorophenyl)propyl-*N*^{1}-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-succinamic acid (compound 236).</u>

General procedure 3.

Starting materials: 2(R)-(4-chlorophenyl)propyl-succinic acid-4-*tert*-butyl ester and L-thionovaline *N*-[2-(2-methoxy-ethoxymethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.7, 176.1, 174.7, 140.3, 131.5, 129.8, 128.4, 95.8, 71.9, 67.1, 65.7, 64.4, 59.0, 45.8, 42.9, 37.3, 34.9, 33.7, 32.0, 28.6, 19.4, 18.6.

<u>Preparation 37: 2(R)-(4-chlorophenyl)propyl-*N*^{1}-[1(S)-(methylthio-carbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamic acid (compound 237).</u>

General procedure 3.

Starting materials: 2(R)-(4-chlorophenyl)propyl-succinic acid-4-*tert*-butyl ester and L-thionotryptophane *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR (DMSO-d_6) δ 203.6, 173.9, 173.8, 141.0, 135.9, 130.0, 127.9, 127.2, 123.5, 120.7, 118.3, 118.0, 111.2, 110.3, 59.8, 42.1, 37.8, 34.2, 32.1, 31.6, 30.4, 28.1.

Preparation 38: N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethyl-thiocarbamoyl]-2-methyl-propyl}-2(R)-(4-methylphenoxy)ethyl-succinamic acid (compound 238).

General procedure 3.

Starting materials: 2(R)-(4-methylphenoxy)ethyl-succinic acid-4-*tert*-butyl ester and L-thionovaline *N*-[2-(2-methoxy-ethoxymethoxy)-ethyl]amide (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.5, 175.4, 174.2, 156.4, 130.2, 129.9, 114.6, 95.8, 71.9, 67.0, 65.9, 65.3, 64.6, 59.0, 45.9, 39.5, 36.4, 33.8, 31.9, 20.5, 19.4, 18.5.

Preparation 39: N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthio-carbamoyl}-(4-methoxyphenyl)ethyl)-2(R)-(4-methylphenoxy)ethyl-succinamic acid (compound 239).

General procedure 3.

Starting materials: 2(R)-(4-methylphenoxy)ethyl-succinic acid-4-*tert*-butyl ester and L-thiono-4-methoxy-phenylalanine *N*-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}amide (prepared as described for compound 205).

^{1}H NMR ($CDCl_3$) δ 8.39(t,1H), 7.11 (d,2H), 7.04 (d,2H), 6.92 (d,1H), 6.79(d,2H), 6.73 (d,2H), 4.80 (q,1H), 3.88 (t,2H), 3.75(s,3H), 3.8-3.4 (m,12H), 3.36 (s,3H), 3.13 (d,2H), 2.92(m,1H), 2.71 (dd,1H), 2.59 (dd,1H), 2.27 (s,3H), 2.00 (m,2H).

Preparation 40: *N^1*-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-2(R)-(4-methylphenoxy)ethyl-succinamic acid (compound 240).

General procedure 3.

Starting materials: 2(R)-(4-methylphenoxy)ethyl-succinic acid-4-*tert*-butyl ester and L-thionotryptophane *N*-methylamide hydrochloric acid salt (prepared as described for compound 205).

^{13}C NMR ($CDCl_3$) δ 203.2, 177.6, 175.7, 156.2, 136.1, 130.4, 130.0, 127.4, 123.6, 122.1, 119.5, 118.8, 114.5, 111.5, 110.2, 65.6, 60.3, 40.3, 38.6, 32.6, 31.9, 31.3, 20.4.

Example 1: *N^4*-Hydroxy-2(R)-phenylethyl-*N^1*-[1(S)-(3-phenylpropyl-thiocarbamoyl)-2-phenylethyl]-succinamide (compound 101).

General procedure 7.

Starting material: compound 209.

^{13}C NMR (DMSO-d_6) δ 202.7, 173.2, 167.4, 141.9, 141.4, 137.6, 129.1, 128.2, 128.1, 128.1, 127.9, 126.2, 125.6, 125.5, 60.0, 44.4, 41.4, 34.6, 33.5, 32.4, 32.3, 28.6.

Example 2: *N^4*-Hydroxy-2(R)-isobutyl-*N^1*-[1(S)-(3-phenylpropyl-thiocarbamoyl)-2-phenylethyl]-succinamide (compound 102).

General procedure 7.

Starting material: compound 210.

^{13}C NMR (DMSO-d_6) δ 202.5, 173.5, 167.4, 141.4, 137.7, 129.0, 128.1, 127.9, 126.1, 125.7, 59.9, 44.4, 40.5, 40.2, 35.6, 32.2, 28.6, 25.0, 23.2, 21.8.

Example 3: *N^4*-Hydroxy-2(R)-isobutyl-*N^1*-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-succinamide (compound 103).

General procedure 7.

Starting material: compound 211.

^{13}C NMR ($CDCl_3$) δ 202.7, 174.9, 168.9, 136.5, 129.2, 128.6, 127.0, 60.3, 42.0, 41.4, 36.2, 32.7, 25.7, 22.9, 22.1.

Example 4: *N*^{4}-Hydroxy-*N*^{1}-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-2(R)-phenylpropyl -succinamide (compound 104).

General procedure 7.

Starting material: compound 212.

^{13}C NMR (DMSO-d_6) δ 203.1, 173.4, 167.6, 142.0, 137.9, 128.9, 128.1, 128.0, 127.9, 126.2, 125.5, 60.1, 41.8, 40.1, 35.0, 34.7, 32.0, 31.3, 28.3.

Example 5: *N*^{4}-Hydroxy-2(R)-phenylpropyl-*N*^{1}-[1(S)-(3-phenylpropyl-thiocarbamoyl)-2-phenylethyl]-succinamide (compound 105).

General procedure 7.

Starting material: compound 213.

^{13}C NMR ($CDCl_3$) δ 201.5, 174.6, 169.0, 141.7, 141.0, 136.3, 129.3, 128.6, 128.4, 128.3, 128.3, 127.1, 126.0, 125.9, 60.7, 45.3, 43.1, 41.5, 35.5, 35.3, 32.9, 32.1, 28.8, 28.6.

Example 6: *N*^{4}-Hydroxy-2(R)-phenylpropyl-*N*^{1}-[1(S)-(3-phenylpropyl-thiocarbamoyl)-2-cyclohexylethyl]-succinamide (compound 106).

General procedure 7.

Starting material: compound 214.

^{13}C NMR ($CDCl_3$) δ 203.8, 174.9, 169.0, 141.6, 141.2, 128.5, 128.4, 126.1, 125.9, 57.2, 45.5, 43.0, 42.6, 35.5, 34.0, 33.3, 33.2, 33.0, 32.4, 29.2, 28.7, 26.4, 26.1, 25.9.

Example 7: *N*^{4}-Hydroxy-*N*^{1}-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-2(R)-phenylpropyl-succinamide (compound 107).

General procedure 7.

Starting material: compound 215.

^{13}C NMR (DMSO-d_6) δ 204.8, 173.5, 167.6, 142.0, 128.1, 128.0, 125.5, 56.5, 42.0, 41.8, 35.1, 34.8, 33.5, 33.1, 32.0, 31.6, 31.6, 28.4, 26.0, 25.7, 25.4.

Example 8: N^4-Hydroxy-2(R)-isobutyl-N^1-thiono- N^1-[1(S)-(methyl-carbamoyl)-2-phenylethyl]-succinamide (compound 108).

General procedure 7.

Starting material: compound 216.

MS [M-H]$^-$ 364, [M-OH]$^-$ 348,[M-NH_2OH]$^-$ 331.

Example 9: 3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthio-carbamoyl)-2-cyclohexylethyl]-succinamide (compound 109).

General procedure 8.

Starting material: compound 217.

^{13}C NMR ($CDCl_3$) δ 204.2, 174.5, 169.8, 71.2, 57.3, 47.2, 42.9, 38.9, 34.4, 33.7, 33.0, 32.3, 26.3, 26.2, 26.0, 25.8, 23.0, 22.1.

Example 10: 3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthio-carbamoyl)-2-phenylethyl]-succinamide (compound 110).

General procedure 8.

Starting material: compound 218.

MS [MH]$^+$ 382, [MNa]$^+$ 404, [MH-NH_2OH]$^+$ 349.

Example 11: N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-phenylpropyl-thiocarbamoyl)-2-cyclohexylethyl]-succinamide (compound 111).

General procedure 7.

Starting material: compound 219.

^{13}C NMR (CD_3OD) δ 205.9, 176.7, 170.6, 142.9, 129.5, 129.4, 127.0, 58.3, 46.1, 43.9, 42.6, 42.4, 37.1, 35.5, 34.7, 34.3, 33.9, 30.6, 27.7, 27.4, 27.2, 26.9, 23.8, 22.4.

Example 12: N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-cyclohexylethyl]-succinamide (compound 112).

General procedure 7.

Starting material: compound 220.

^{13}C NMR (CD_3OD) δ 206.6, 176.8, 58.2, 43.9, 42.7, 37.1, 35.5, 34.9, 33.6, 32.7, 27.7, 27.4, 27.2, 26.9, 23.8, 22.4, 15.5.

Example 13: N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide (compound 113).

General procedure 7.

Starting material: compound 221.

^{13}C NMR (DMSO-d_6) δ 204.4, 174.4, 168.4, 136.8, 128.1, 124.4, 121.6, 119.2, 118.9, 112.0, 111.0, 60.5, 41.4, 36.3, 33.0, 31.4, 25.8, 23.9, 22.8.

Example 14: N^4-Hydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-phenylethyl}-2(R)-phenylpropyl-succinamide (compound 114).

General procedure 7.

Starting material: compound 222.

^{13}C NMR ($CDCl_3$) δ 202.2, 174.1, 141.7, 136.6, 129.3, 128.5, 128.4, 128.3, 126.9, 125.9, 95.6, 71.8, 66.9, 65.4, 60.7, 58.9, 45.8, 43.3, 41.8, 35.5, 32.0, 29.7, 28.7.

Example 15: 3(S), N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthio-carbamoyl)-2,2-dimethyl-propyl]-succinamide (compound 115).

General procedure 8.

Starting material: compound 223.

^{13}C NMR (DMSO-d_6) δ 201.7, 171.6, 168.8, 71.6, 64.3, 48.3, 35.2, 31.8, 26.9, 26.8, 25.2, 23.6, 21.7.

Example 16: 3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2,2-dimethyl-propyl}-succinamide (compound 116).

General procedure 9.

Starting material: compound 224.

^{13}C NMR (DMSO-d_6) δ 201.6, 173.0, 169.2, 135.9, 116.2, 71.2, 69.4, 67.1, 64.6, 58.1, 46.3, 45.9, 44.7, 34.9, 34.5, 26.9, 25.1, 24.2, 21.7.

Example 17: 3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide (compound 117).

General procedure 9.

Starting material: compound 225.

^{13}C NMR (DMSO-d_6) δ 204.4, 172.8, 169.2, 157.7, 135.8, 130.3, 129.5, 115.5, 113.2, 71.1, 69.4, 67.1, 60.0, 58.0, 54.6, 45.9, 45.6, 44.9, 34.2, 24.9, 24.2, 21.5.

Example 18: N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-methyl-propyl]-3(S)-propyl-succinamide (compound 118).

General procedure 9.

Starting material: compound 226.

^{13}C NMR (DMSO-d_6) δ 204.1, 173.2, 170.0, 64.4, 46.1, 45.7, 32.7, 31.7, 31.5, 25.0, 24.1, 21.6, 19.9, 19.2, 18.9, 13.8.

Example 19: N^4-Hydroxy-2(R)-isobutyl-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-3(S)-propyl-succinamide (compound 119).

General procedure 9.

Starting material: compound 227.

^{13}C NMR (DMSO-d_6) δ 206.0, 173.8, 170.4, 71.7, 70.1, 70.0, 67.5,58.5, 57.3, 46.6, 46.3, 45.3, 44.0, 33.3, 25.5,24.7, 23.6, 22.0, 20.5, 14.4.

Example 20: 2(R)-Dodecyl-N^4-hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-3-methyl-butyl]-succinamide (compound120).

General procedure 7.

Starting material: compound 228.

^{13}C NMR ($CDCl_3$) δ 204.7, 175.0, 169.3, 57.6, 43.8, 43.5, 33.1, 32.7, 31.9, 29.7, 29.4, 27.2, 24.9, 22.9, 22.7, 22.2, 14.1.

Example 21: 2(R)-Dodecyl-N^4-hydroxy-N^1-[1(S)-(phenylethylthiocarbamoyl)-2-methyl-butyl]-succinamide (compound 121).

General procedure 7.

Starting material: compound 229.

^{13}C NMR (DMSO-d_6) δ 203.5, 173.5, 167.5, 138.8, 128.4, 128.2, 126.1, 62.7, 46.0, 41.4, 37.8, 34.9, 32.8, 31.8, 31.2, 29.1, 29.0, 28.9, 28.9, 28.9, 28.6, 26.4, 24.2, 22.0, 15.1, 13.8, 10.7.

Example 22: 2(R)-Hexadecyl-N^4-hydroxy-N^1-[1(S)-(phenylthiocarbamoyl)-ethyl]-succinamide (compound 122).

General procedure 7.

Starting material: compound 230.

^{13}C NMR (DMSO-d_6) δ 204.9, 173.7, 167.7, 139.4, 128.4, 125.9, 122.8, 55.6, 41.5, 34.8, 32.0, 31.3, 29.1, 28.7, 26.5, 22.1, 21.0, 14.0.

Example 23: 2(R)-Hexadecyl-N^4-hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamide (compound 123).

General procedure 7.

Starting material: compound 231.

^{13}C NMR (DMSO-d_6) δ 201.7, 173.3, 167.4, 64.3, 41.6, 35.0, 34.7, 31.7, 31.6, 31.2, 29.0, 28.8, 28.6, 26.7, 26.4, 22.0, 13.8.

Example 24: 3(S), N^4-Dihydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)-ethyl}-2(R)-phenylpropyl-succinamide (compound 124).

General procedure 8.

Starting material: compound 232.

^{13}C NMR ($CDCl_3$) δ 201.9, 173.4, 169.6, 158.6, 141.7, 130.3, 128.4, 128.4, 125.9, 113.9, 72.2, 71.7, 69.9, 68.2, 61.3, 58.9, 55.2, 48.4, 45.5, 40.3, 35.5, 29.4, 28.7.

Example 25: 3(S), N^4-Dihydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-phenylpropyl-succinamide (compound 125).

General procedure 8.

Starting material: compound 233.

^{13}C NMR (DMSO-d_6) δ 203.1, 174.1, 169.6, 141.7, 128.4, 128.4, 125.9, 95.7, 72.3, 71.9, 67.0, 65.6, 64.8, 59.0, 47.8, 45.8, 35.5, 33.3, 29.5, 28.8, 19.4, 18.4.

Example 26: N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide (compound 126).

General procedure 7.

Starting material: compound 234.

^{13}C NMR (DMSO-d_6) δ 203.6, 173.3, 167.5, 157.7, 141.0, 130.1, 130.0, 129.5, 127.9, 113.3, 71.1, 69.3, 66.9, 60.1, 58.0, 54.8, 44.7, 41.5, 34.7, 34.2, 31.0, 28.1.

Example 27: N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-succinamide (compound 127).

General procedure 7.

Starting material: compound 235.

^{13}C NMR (DMSO-d_6) δ 205.0, 173.4, 167.5, 141.0, 130.0, 127.9, 71.2, 69.6, 69.5, 66.9, 58.0, 57.1, 44.7, 43.6, 41.4, 34.8, 34.2, 31.3, 28.2, 24.1, 22.8, 21.6.

Example 28: *N*[4]-Hydroxy-2(R)-(4-chlorophenyl)propyl-*N*[1]-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-succinamide (compound 128).

General procedure 7.

Starting material: compound 236.

^{13}C NMR (DMSO-d_6) δ 203.9, 173.4, 167.5, 141.0, 130.0, 127.9, 94.6, 71.1, 66.2, 64.0, 57.9, 44.7, 41.1, 34.9, 34.2, 32.2, 31.3, 28.1, 19.1, 18.5.

Example 29: *N*[4]-Hydroxy-2(R)-(4-chlorophenyl)propyl-*N*[1]-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide (compound 129).

General procedure 7.

Starting material: compound 237.

^{13}C NMR (DMSO-d_6) δ 203.7, 173.4, 167.6, 141.0, 135.9, 130.0, 127.9, 127.2, 123.6, 120.7, 118.3, 118.1, 111.2, 110.1, 59.7, 41.8, 34.7, 34.2, 32.1, 31.2, 30.5, 28.1.

Example 30: *N*[4]-Hydroxy-*N*[1]-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-(4-methylphenoxy)ethyl-succinamide (compound 130).

General procedure 7.

Starting material: compound 238.

^{13}C NMR ($CDCl_3$) δ 203.5, 174.0, 168.4, 156.4, 130.2, 129.9, 114.6, 95.8, 71.9, 67.1, 65.8, 65.5, 65.0, 59.0, 45.8, 40.5, 35.4, 33.5, 32.2, 20.5, 19.4, 18.6.

Example 31: N^4-Hydroxy-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-(4-methoxyphenyl)ethyl)-2(R)-(4-methylphenoxy)ethyl-succinamide (compound 131).

General procedure 7.

Starting material: compound 239.

^{13}C NMR ($CDCl_3$) δ 202.5, 173.8, 168.7, 158.5, 156.4, 130.3, 130.1, 129.9, 128.8, 114.5, 113.9, 71.9, 70.4, 70.3, 70.1, 68.2, 65.4, 61.7, 58.9, 55.2, 45.4, 40.4, 40.3, 35.3, 31.8, 20.5.

Example 32: N^4-Hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-2(R)-(4-methylphenoxy)ethyl-succinamide (compound 132).

General procedure 7.

Starting material: compound 240.

^{13}C NMR (DMSO-d_6) 203.7, 172.9, 167.3, 156.2, 135.9, 129.6, 128.9, 127.2, 123.7, 120.7, 118.4, 118.1, 114.1, 111.2, 110.0, 65.3, 59.8, 34.8, 32.1, 31.0, 30.5, 20.0.

Example 33: Capsules containing compound 103.

Compound 103 was dissolved in fractionated coconut oil to a final concentration of 10 mg/ml. Ten parts by weight of gelatine, 5 parts by weight of glycerin, 0.08 parts by weight of potassium sorbate, and 14 parts by weight of distilled water were mixed together with heating and formed into soft gelatine capsules. These were then filled each with 500 µl of the oily solution of compound 103.

Example 34: Tablet containing compound 103.

Compound 103	
(active substance)	50 mg
Lactose	125 mg
Starch	12 mg

Methyl cellulose	2 mg
Sodium carboxymethyl cellulose	10 mg
Magnesium stearate	1 mg

The active substance, lactose and starch are mixed to a homogeneous state in a suitable mixer and moistened with a 5 per cent aqueous solution of methyl cellulose 15 cps. The mixing is continued until granules are formed. If necessary, the wet granulation is passed through a suitable screen and dried to a water content of less than 1% in a suitable drier, e.g. fluid bed or drying oven. The dried granules are passed through a 1 mm screen and mixed to a homogeneous state with sodium carboxymethyl cellulose. Magnesium stearate is added, and the mixing is continued for a short period of time.

Tablets with a weight of 200 mg are produced from the granulation by means of a suitable tabletting machine.

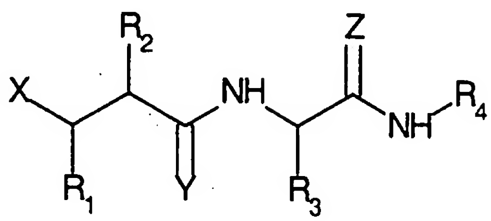
Example 35: Formulation for injection containing compound 103.

Compound 103	
(active substance)	1%
Sodium chloride	q.s.
Ethanol	10%
Water for injection to make	100%

The active substance is dissolved in ethanol (10%) then water for injection made isotonic with sodium chloride is added to make 100%. The mixture is filled into ampoules and sterilized.

WHAT WE CLAIM IS:

1. A compound of the general formula (I)



(I)

wherein X is a $-CO_2H$ or -CONHOH group; Y and Z are independently sulphur or oxygen, at least one being sulphur; R_1 is hydrogen, hydroxy, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, or (C_3-C_8)cycloalkyl; R_2 is a (C_1-C_{24})alkyl, phenyl(C_1-C_6)alkyl, or phenyl$(C_0-C_6$ alkyl$)O(C_1-C_6)$alkyl, any of which may be optionally substituted with (C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo, or cyano (CN); R_3 is the characterising side chain of a natural α-amino acid in which any functional groups may be protected, (C_1-C_6)alkyl which may be optionally substituted, or cycloalkyl(C_1-C_6)alkyl; R_4 is hydrogen, (C_1-C_6)alkyl, phenyl(C_1-C_6)alkyl, optionally substituted phenyl or heteroaryl, or a group of formula $-(Q-O)_n-Q$ where Q is (C_1-C_6)alkyl and where n is an integer >1, and no continuous linear sequence of atoms in the group R_4 is >12; any of the above alkyl or alkenyl groups being straight or branched; or a salt, hydrate or solvate thereof.

2. A diastereoisomer of a compound according to claim 1, in pure form; or a mixture of stereoisomers of a compound according to claim 1.

3. A compound according to any one of the preceding claims wherein R_1 is hydrogen, hydroxyl, allyl or propyl.

4. A compound according to any one of the preceding claims wherein R_2 is isobutyl, phenylpropyl, (4-chlorophenyl)propyl, (4-methylphenoxy)ethyl or (C_6-C_{16})alkyl.

5. A compound according to any one of the preceding claims wherein R_3 is benzyl, t-butyl, cyclohexylmethyl, 4-methoxybenzyl, indolmethyl, isobutyl and isopropyl.

6. A compound according to any one of the preceding claims wherein R_4 is methyl, phenylpropyl, 2-(2-methoxyethoxy)ethyl, 2-(2-methoxyethoxy-methoxy)ethyl or 2-(ethoxyethoxymethoxy)ethyl.

7. A compound according to claim 1 wherein R_1 is hydrogen, hydroxyl, allyl, or propyl; R_2 is isobutyl, phenylpropyl, (4-chlorophenyl)propyl, (4-methylphenoxy)ethyl, or (C_6-C_{16})alkyl; R_3 is benzyl, t-butyl, cyclohexylmethyl, 4-methoxybenzyl, indolmethyl, isobutyl, or isopropyl; R_4 is methyl, phenylpropyl, 2-(2-methoxyethoxy)ethyl, 2-(2-methoxyethoxymethoxy)ethyl, or 2-(ethoxyethoxymethoxy)ethyl.

8. A compound according to the preceding claims which is selected from the group consisting of:

a) N^4-Hydroxy-2(R)-phenylethyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide,

b) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide,

c) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-phenyl–ethyl]-succinamide,

d) N^4-Hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-2(R)-phenylpropyl]-succinamide,

e) N^4-Hydroxy-2(R)-phenylpropyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-phenylethyl]-succinamide,

f) N^4-Hydroxy-2(R)-phenylpropyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamide,

g) N^4-Hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-2(R)-phenylpropyl-succinamide,

h) N^4-Hydroxy-2(R)-isobutyl-N^1-thiono- N^1-[1(S)-(methylcarbamoyl)-2-phenylethyl]-succinamide,

i) 3(S),N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-cyclohexylethyl]-succinamide,

j) 3(S),N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-phenylethyl]-succinamide,

k) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-phenylpropylthiocarbamoyl)-2-cyclohexylethyl]-succinamide,

l) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-cyclohexylethyl]-succinamide,

m) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(3-methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide,

n) N^4-Hydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-phenylethyl}-2(R)-phenylpropyl-succinamide,

o) *3(S)*,N^4-Dihydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamide,

p) 3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2,2-dimethyl-propyl}-succinamide,

q) 3(S)-Allyl-N^4-hydroxy-2(R)-isobutyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide,

r) N^4-Hydroxy-2(R)-isobutyl-N^1-[1(S)-(methylthiocarbamoyl)-2-methyl-propyl]-3(S)-propyl-succinamide,

s) N^4-Hydroxy-2(R)-isobutyl-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-3(S)-propyl-succinamide,

t) 2(R)-Dodecyl-N^4-hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-3-methyl-butyl]-succinamide,

u) 2(R)-Dodecyl-N^4-hydroxy-N^1-[1(S)-(phenylethylthiocarbamoyl)-2-methyl-butyl]-succinamide,

v) 2(R)-Hexadecyl-N^4-hydroxy-N^1-[1(S)-(phenylthiocarbamoyl)-ethyl]-succinamide,

w) 2(R)-Hexadecyl-N^4-hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2,2-dimethyl-propyl]-succinamide,

x) 3(S),N^4-Dihydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)-ethyl}-2(R)-phenylpropyl-succinamide,

y) 3(S), N^4-Dihydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-phenylpropyl-succinamide,

z) N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-{1(S)-[2-(2-methoxy-ethoxy)-ethylthiocarbamoyl]-2-(4-methoxyphenyl)ethyl}-succinamide,

aa) N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-3-methyl-butyl)-succinamide,

bb) N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-succinamide,

cc) N^4-Hydroxy-2(R)-(4-chlorophenyl)propyl-N^1-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-succinamide,

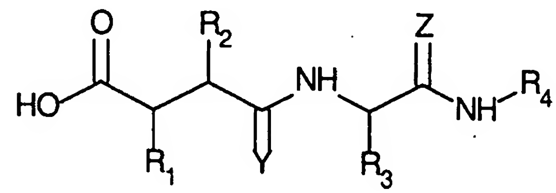
dd) N^4-Hydroxy-N^1-{1(S)-[2-(2-methoxy-ethoxymethoxy)-ethylthiocarbamoyl]-2-methyl-propyl}-2(R)-(4-methylphenoxy)ethyl-succinamide,

ee) N^4-Hydroxy-N^1-(1(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylthiocarbamoyl}-(4-methoxyphenyl)ethyl)-2(R)-(4-methylphenoxy)ethyl-succinamide;

ff) N^4-Hydroxy-N^1-[1(S)-(methylthiocarbamoyl)-2-(1*H*-indol-3-yl)ethyl]-2(R)-(4-methylphenoxy)ethyl-succinamide;

a diastereoisomer of any one of said compounds in pure form; a mixture of stereoisomers of any one of said compounds; and a pharmaceutically acceptable salt, hydrate, or solvate of any one of said compounds.

9. A method for producing a compound of formula I of claim 1 by which an acid of general formula (II)



(II)

is reacted with hydroxylamine, O-protected hydroxylamine, or N,O-diprotected hydroxylamine, the acid of formula (II) possibly protected from said reaction, whereafter removing any protecting groups from the resulting hydroxamic acid moiety and from any protected substituents in R_1, R_2, R_3, and R_4.

10. A pharmaceutical composition containing an effective amount of one or more of the compounds of claims 1-8 as an active ingredient, together with pharmaceutically acceptable carriers and/or auxiliary agents.

11. A pharmaceutical composition according to claim 10 in dosage unit form for systemic treatment containing from 0.07 mg to 1 g of one or more of the compounds of claims 1-8 as an active ingredient.

12. A pharmaceutical composition according to claim 10 in dosage unit form containing from 0.5 mg to about 500 mg of one or more of the compounds of claims 1-8 as an active ingredient.

13. A method for the treatment or prophylaxis of conditions involving tissue breakdown and inflammation, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration, and tumour metastasis, invasion and growth, and for the treatment of neuroinflammatory disorders, including those involving myelin degradation, for example multiple sclerosis, as well as for the management of angiogenesis dependent diseases, which include arthritic conditions and solid tumour growth as well as psoriasis, proliferative retinopathies, neovascular glaucoma, ocular tumours, angiofibromas and hemangiomas, consisting in administering to a patient in need thereof a pharmaceutical composition according to claim 10, 11 or 12.

14. The use of a compound according to any one of claims 1-8 in the manufacture of a medicament for the treatment or prophylaxis of conditions involving tissue breakdown and inflammation, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration, and tumour metastasis, invasion and growth, and for the treatment of neuroinflammatory disorders, including those involving myelin degradation, for example multiple sclerosis, as well as for the management of angiogenesis dependent diseases, which include arthritic conditions and solid tumour growth as well as psoriasis, proliferative retinopathies, neovascular glaucoma, ocular tumours, angiofibromas and hemangiomas.

INTERNATIONAL SEARCH REPORT

Inte ...ional Application No
PCT/DK 99/00072

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C327/42 C07C327/44 C07D209/20 A61K31/16 A61K31/195
A61K31/655

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 214 639 A (SEARLE) 18 March 1987 cited in the application see page 2 - page 6 ---	1,10
A	EP 0 489 579 A (CELLTECH) 10 June 1992 cited in the application see page 2 ---	1,10
A	WO 96 16931 A (BRITISH BIOTECH PHARMACEUTICALS) 6 June 1996 cited in the application see page 8 -----	1,10

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 May 1999	20/05/1999

Name and mailing address of the ISA	Authorized officer
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